

To Geneva yet again

REPRESENTATIVES of the United States, United Kingdom and Soviet Union are back in Geneva this week to resume talks on a comprehensive ban on nuclear testing. Regular readers of these columns could be excused for stifling a slight yawn on hearing this; for many years now it has seemed to us and to others that a ban was just around the corner. Each year we have been reliably advised that the odds were fifty-fifty on a treaty by Christmas, but those who believe that this implies ultimate success should ponder the message of the coin tossed in Tom Stoppard's play *Rosencrantz*—which came down 'heads' more than eighty times in a row.

At present the two superpowers operate a voluntary limit of 150 kilotons on underground tests, and they, along with most other countries, are prevented from firing above ground by the Partial Test-Ban Treaty of 1963. France and China, non-signatories of the treaty, used to test regularly in the atmosphere, but in recent years, they too have gone underground. Although only three countries are party to the present negotiations on a comprehensive treaty, it is hoped that if and when a document is produced it will prove widely acceptable, and even those seasoned non-signers, France and China, will be prepared to adhere to its spirit. It goes almost without saying that many nations under strong pressure not to venture into nuclear proliferation are very keen indeed for some evidence that the superpowers are putting a brake on their own weapons programmes, and a comprehensive test ban, although only a partial dampener on weapons development, has great symbolic importance.

What then holds up a treaty? The traditional reason for delay has been the inadequacy of systems for monitoring compliance, whether by seismic or other means. There has been considerable progress in these matters in the past ten years; further major improvements in the near future are, however, unlikely. Above certain yields the risk of detection and identification is now very high, but below these levels there is more and more scope for undetectable clandestine activity. No technical monitoring system can provide complete satisfaction. Fortunately this now seems to be widely understood; no interested party would now be at liberty suddenly to 'discover' inadequacies in the monitoring system and claim that negotiations had been conducted in ignorance of them.

A more recent obstacle to a treaty has been peaceful nuclear explosions. The almost complete loss of support for these in the United States, on economic and public-acceptability grounds, contrasts with enthusiasm within the Soviet Union, where more than forty such explo-

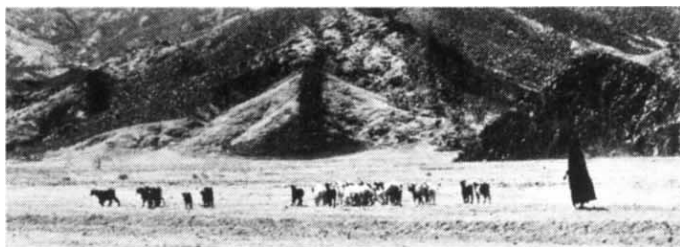
sions have been fired for a variety of purposes. But even in the Soviet Union the pace seems to have slackened off of late, and elsewhere no one—not even the Indians—now has much enthusiasm. The trouble with peaceful explosions is that there is absolutely no way of verifying that they do not also have a military purpose, and any attempt to exclude such explosions from a comprehensive test-ban would have to be viewed with the utmost suspicion.

Most recently, concern has been expressed in the United States about the effects that a test-ban would have on stockpile efficiency; it is claimed that in the absence of regular testing, devices cannot be trusted indefinitely. This is a controversial matter; not even all those associated with the weapons laboratories would have the same point of view. But there have already been calls for the ban not to be total, but to allow limited low-yield testing. If peaceful explosions could be a vehicle for weapon development, however, so too could ostensible stockpile testing.

So what will emerge from Geneva? It has to be acceptable to the US Senate which ratifies such international agreements, so it will probably err on the side of caution. The Soviet Union seems prepared to forego peaceful explosions, but only for a short period. A limited duration treaty would suit the United States fine, because it would sidestep the question of the future of the weapons laboratories. At a guess there will be a three year ban on the firing of any nuclear devices whatsoever. But do not be deceived; although the treaty will undoubtedly come up for renewal there must be the most serious doubts that it actually will be renewed. Between 1958 and 1961 there was a thirty-four month moratorium in testing but it did not lead to a permanent ban. From public pronouncements in the United States and from an apparent Soviet desire to push ahead on the peaceful uses front it seems clear that many have already made up their mind that the ban will merely be a temporary measure.

What purpose does a British presence serve at these talks? Undoubtedly it has a certain cosmetic value in dispelling the impression that the two superpowers are colluding, and probably there are technical matters where British representation injects a sensible new dimension. But Britain is so closely tied to the United States in nuclear affairs that it is most unlikely that a truly independent line could be taken. It is probable that the treaty which will emerge from Geneva will be a poor substitute for a truly comprehensive permanent ban; somewhere an independent powerful and respected voice should be saying this. □





Sinai in peace and in war: one plan was to make it an Arab-Israeli "national park"

Israel wants scientific cooperation with the Arabs

ISRAEL scientists see the outcome of the Camp David talks as a unique challenge and chance for mutual cooperation—a result which may, at first, seem paradoxical, since the settlement will mean handing over to Egyptian control one of the most strictly protected conservation areas in the world.

During the 12 years that Sinai has been under Israeli control, environmental restrictions have been so strict that not even a single shell could legally be removed from the beach without special permission. The fragile ecology of the area has been extensively studied, and investigations of the "solar lake", a few kilometres south of the pre-1967 frontier have provided a new concept in the commercial use of solar energy. For this lake has a natural stratification of salinity allowing bottom water temperature to build up to more than 60 °C, without convective heating of the upper layers which remain at approximately 20 °C. Not surprisingly, at the international conference on the application of solar energy held earlier this month at the Technion—Israel's institute of technology—there was a whole session devoted to artificial solar ponds, which, it is hoped, will ultimately be used for electricity generation, desalination of sea-water and for low temperature industrial heat production.

As far as the ecology of the Sinai is concerned, the non-governmental Society for the Protection of Nature, which has maintained three field study centres in the Sinai—at Na'ama, Yamit, and Santa Katarina—is willing to extend to the Egyptians any assistance they may require. Indeed, Professor Amotz Zahavi told *Nature* that several years ago, the society suggested that the Sinai should be made into an "international park for peace" under joint Israeli/Egyptian control.

For some years, said Professor Zahavi, the society has been passing on its findings and ideas on conservation to the Israeli authorities. Now, with the hand-over to the Egyptians, their experts would be willing to stay on at the field centres, working in collaboration with the Egyptians for the sake of conservation. Professor Zahavi stressed

that the Israeli teams have no idea how the Egyptians will react to this proposal. "So far as we know, conservation is not yet very well developed in Egypt," he said, a further reason why his society is so concerned to help.

Israel has an impressive record in desert reclamation and the cultivation of marginal lands. With perhaps one-third of the world's cultivable land threatened with desertification, Israeli environmentalists describe as tragic the Arab walk-out from last year's conference on desertification when an Israeli delegate rose to speak.

At Haifa, the Kogan-Rose desalination pilot plant, which uses a counter-flow system to eliminate the need for expensive copper-tubing heat-exchangers, has proved its worth, and plans are underway to construct a full-scale installation capable of delivering some million cubic metres per day. The main question to be decided now is where to build it. While such questions are, of course, matters for the politicians to decide, Professor Abraham Kogan, the designer, speaks of a possible joint Israeli-Egyptian venture.

Indeed, the consensus of Israeli scientific opinion seems to run ahead of the formal political situation. Last summer, in his report to the international board of governors of the Technion, made on the occasion of the 30th anniversary of Israeli independence, the Technion president, Amos Horev, stressed that technological cooperation "could well be put into effect with Arab neighbours even in advance of formal peace". Ecologists and technologists are already looking hopefully towards other frontiers. The Dead Sea potash works, for example, which produces fertilizers from evaporated Dead Sea brines, must use a certain amount of non-saline water in the extraction process. With a growing world demand for fertilizer and a virtually inexhaustible supply of raw material, it is small wonder that some engineers think longingly of the fresh water supply, just across the Jordanian frontier, which in British Mandate times was actually piped to the forerunner of the present potash works (then sited at the northern end of the Dead Sea).

Jordan is known to be considering a potash works of her own—a joint development would be more economical and also a powerful factor in the continued peace of the area.

Likewise, marine ecologists in the Gulf of Aqaba stress that however carefully they monitor the tankers discharging at the Eilat oil terminal, the gulf is narrow, and has no real tidal scour. A joint protection and monitoring programme involving all the littoral states—Israel, Jordan, Saudi Arabia, and Egypt—is the best hope of avoiding major pollution in this part of the gulf.

And, looking even further ahead, Professor Y. Avnimelech of the Kinneret water research project even mentioned the old Johnstone plan of Mandate times, by which the river Yarmuk would be diverted into Kinneret, the augmented waters of which could then be used for the benefit of what now constitutes both Israel and Jordan. Since the Kinneret now serves as the sole fresh water source for Israel's national water grid, and the Israeli water economy is so tight that brackish and waste water irrigation is an increasing necessity, even the most academic allusion to such a scheme reflects a considerable commitment to the idea of cooperation.

Such a commitment, however, is more than a pipe-dream of a few scientists, however far ahead the realisation may be, the Israeli government undoubtedly endorses the underlying principle. Addressing the international congress of ecology, which was held in Jerusalem during the week of the Camp David talks, Mr Yigal Yadin, the Israeli deputy prime minister, stressed that "ecology has no national boundaries". After outlining Israel's considerable achievement in developing the country without major ecological damage, while at the same time extending aid to such third world countries as would accept it, he concluded: "We are yearning for peace so that we can strive for a better world. Let us endeavour when peace comes on earth—if the earth still be there—we may be more worthy to enjoy it".

Vera Rich

Argentina: concern over continued disappearances

On the eve of the Twelfth International Cancer Congress in Buenos Aires, David Dickson reports on the fate of 15,000 missing Argentinians, many of them scientists

Two weeks ago Dr Lepanto Bianchi, an Argentinian orthopaedic surgeon, was arrested and taken from his office by men who claimed that they were from the federal police force. Dr Bianchi's arrest follows the similar disappearance last month of Beatriz Perioso, president of the Argentine Federation of Psychologists, who was taken away from a child care centre at which she had been working.

No news has since been received of Dr Perioso's whereabouts, nor whether any charges have been made against her. And neither has any information been received about two other psychologists, Arturo Smith and his wife Celia Kriado, who were taken from their home the following day.

The American Psychological Association has written to the Argentinian President, General Jorge H. Videla, requesting information about the situation of the three psychologists, but no reply has been received.

Professor freed

Meanwhile, however, the Argentine authorities last week released Dr Claudio Bermann, a former professor of psychiatry at the University of Cordoba and director of a psychiatric clinic, who had been arrested and held without specific charges since April 1976.

A number of foreign institutions, including Amnesty International and the US National Academy of Sciences' Committee on Human Rights, as well as the Buenos Aires-based Permanent Assembly on Human Rights, had pressed the Argentine government for his release. Last week he was given permission to leave the country, and flew directly to Israel.

In August Elena Sevilla, a research physicist at the Universidad del Sur in Bariloche who had been arrested in November 1975 in hospital shortly after giving birth, was allowed to leave the country after similar moves had been made on her behalf and is now in the US. However in the same month the Permanent Assembly was notified of 11 more "disappearances", and suspects the actual figure may be four or five times this number.

A number of scientists are planning to raise the issue of human rights at the Twelfth International Cancer Conference, which begins in Buenos Aires next week, and is expected to be attended by 7,000 scientists from all over the world.

The National Academy of Sciences, following a visit to Argentina earlier

this year by three representatives of its committee on human rights, decided not to endorse a proposed boycott of the meeting by US scientists. However the committee agreed to assist "those Congress attendees who so desire to express their human rights concerns while in Argentina," primarily through supporting the activities of the human rights clearinghouse of the American Association for the Advancement of Science.

A number of those attending the conference are planning to hold regular caucus meetings on the human rights situation in Argentina. And facilities are also being made available to provide conference delegates with information about the current situation by the Permanent Assembly.

Conference officials point out that the high expected number of participants indicates that moves to boycott the conference—a parallel conference, for example, is being held in Paris under the sponsorship of Professor Andre Lwoff, and is expected to attract several hundred participants—are likely to have relatively little impact on the Buenos Aires arrangements.

Other Argentine scientists have publicly objected to the "pernicious propaganda" which they claim is being spread about the current situation. "At present, the universities and research institutes are places of quiet and fruitful work, in contrast to the agitation, the activism and the persecution of professor and scientists that were so frequent before March 1976 [the date of the military coup]", according to two members of the Buenos Aires National Institute of Medicine in a recent letter to science.

Academic asphyxia

The Permanent Assembly, which brings together various Argentinian groups concerned with human rights, sees the situation slightly differently. "In the national universities, if a climate of perfect order rules, it has been achieved at the cost of the virtual extinction of all interchange of ideas, and similarly the elimination of the whole of a vast cultural sector whose political ideas do not coincide with those of the university authorities," the assembly says in the most recent report on its activities.

"This situation has brought about a noticeable impoverishment of the academic level, generating a dangerous climate of academic asphyxia," according to the assembly's third commission,

which is responsible for the areas of culture, religion, the arts, the sciences, the professions and technical fields.

Earlier this year the assembly published in the Buenos Aires newspaper *La Prensa* a list of 2,500 names of individuals who had disappeared since March 1976, and whose current whereabouts were unknown. A further 500 cases were reported to the assembly after the advertisement appeared, and officials estimate that they have only been informed of one-fifth of those who have disappeared, giving a total number of 15,000.

Many killed

It is feared that up to half of these—men, women and children—have been killed. In addition, it is estimated that there are 8,000 political prisoners being held in Argentine goals, at least half of whom have not been through a proper judicial process.

Scientists figure prominently among those who have disappeared or been imprisoned, particularly those previously involved in organisations such as the now-defunct Argentine Physical Society, which had taken an active part in debates on political issues in the period immediately before the military coup of 1976. However, other scientists who have disappeared or been imprisoned have, it is claimed, played little part in political activity.

Over 2,000 scientists, including both university teachers and research workers, lost their jobs when the military government came into power. Many have since left the country: "the low salaries, added to the lack of security, threats, and the lack of an adequate scientific programme, has motivated the exodus of a great number of scientists and professionals," according to the assembly's report.

Some Argentinian scientists who remain are also concerned at the low priority given to education in the national budget. "In the early 1960s, expenditure on education represented about 20 per cent of total government spending; now it is down to 8.3 per cent," according to Professor Jose Westerkamp, a nuclear physicist at the University of Buenos Aires.

Some areas of research, such as nuclear engineering, still flourish. But in many fields of more basic science, the combined lack of professional manpower, adequate funds, and postgraduate students has resulted in a dramatic decline in activity. □

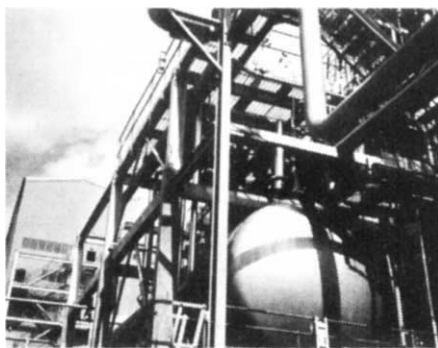
US scientists in new row over cancer at work

PREVIOUS studies of the incidence of cancer associated with occupational exposure to toxic substances may have seriously underestimated the true extent of the problem, according to a statement issued last week by three of the US National Institutes of Health.

Such studies had tended to estimate that the incidence of cancers which would be associated with occupational exposure was between 1 and 15 per cent. But they had been flawed by factors such as the failure to take into account the latency period of several decades that may occur between exposure and the appearance of a cancer, or the synergistic effect of exposure to several carcinogens, the statement claims.

Extrapolating from the results of recent studies of asbestos workers, it concludes that "there is nothing in the gross cancer statistics for the US population which is inconsistent with the hypothesis that up to 20-40 per cent of all cancers are (or will be in the next several decades) attributable to occupational factors".

Those who have contributed to the statement include Dr Arthur Upton, director of the National Cancer Institute, and Dr David Rall, director of the National Institute of Environmental Health Sciences, as well as



PVC production: dispute over hazards

eight other scientists from these two institutes and the National Institute for Occupational Safety and Health.

To offer a figure for comparing the risks from exposure to various industrial compounds, the statement chose "excess cancers" associated with each. (This quantity is defined as the product of the exposed population, the age-adjusted incidence of cancer and an adjusted risk ratio.) The number of annual excess cancers associated with asbestos is then 13,900, but several other substances have excess numbers almost as high: arsenic, 7,300; benzene, 1,400; chromium, 7,900; nickel oxides, 7,300; and petroleum products, 9,100.

The statement predicts that in the years ahead, cancers caused by exposure to other substances will also begin to rise sharply. "The five other agents together pose hazards similar to or greater than those posed by asbestos," says the report. "If only one of the thousands of chemicals introduced into commerce in the past 30

years proves to be as hazardous as asbestos, this could suffice to maintain comparable rates of occupationally-related cancer for decades into the future."

Several industry scientists refused to comment on the report until they had had several weeks to analyse the data. However the President of the Society of the Plastics Industry called the statement "an embarrassment to medical science". He cited a section of the report that predicted 1,940 excess cancer deaths a year to exposure to vinyl chloride as evidence of faulty methodology. "The facts show clearly that vinyl chloride is hazardous to man only at high levels of exposure and over relatively long periods of time", he said. The levels of population considered at risk by the statement are much too high, he concludes, saying that only 23 cases of cancer caused by exposure to vinyl chloride have been confirmed in the last 16 years.

The statement, which is based largely on a number of individual studies carried out by various of its authors, was used by Health Secretary Joseph A. Califano as the basis of a speech earlier this month to a national conference on occupational health organised by the American Federation of Labour-Confederation of Industrial Organisations (AFL-CIO).

In his speech, Dr Califano said that the results were "alarming", and that they would lead to an increased emphasis by the administration on the prevention rather than the cure of cancer.

John Douglas

Radio back-up in rural health service

A VHF communication network is now operating in the rural area of Ballabgarh, 35 kilometres south-east of Delhi. The project is an experiment to see whether such a system is an economic and effective way of providing health care for India.

The network was planned and set up by the Information, Planning and Analysis Group of the Electronics Commission for the All India Institute of Medical Sciences which is running a Comprehensive Rural Health Service Project at Ballabgarh, providing health services to the local community and teaching medical and paramedical staff.

The VHF network consists of four 15-watt fully transistorised transceivers—three fixed and one mobile—and one 400-mW walkie-talkie set. The fixed transceivers are at Ballabgarh Referral Hospital and two primary health centres and the mobile transceiver in a hospital van. The walkie-talkie set is provided to health workers during visits to patients in villages to communicate

with the main hospital. The network covers 368.64 square kilometres and a population of 100,000.

In India, where the majority of people live in villages, health centres are widespread with no telephone links between them. Information about both routine and emergency cases has to be delivered by hand and may often arrive too late to be of any use. A communication network between health centres is therefore seen as essential for effective health care in rural areas, and a VHF communication network is a novel move. The cost-effectiveness has yet to be established. For this purpose, each call made or received from a station is recorded and medical case histories connected with each call pursued to find out the usefulness of the network in treating the patient. If the project proves its worth, its scope could be enlarged and the Ministry of Health and Family Welfare will be asked to establish similar networks in other areas.

Zaka Imam

Scepticism over Soviet promise to let Levich go

FRIENDS and colleagues of Veniamin Levich have greeted with guarded enthusiasm the news that he may shortly receive a visa to emigrate from the USSR to Israel. This is not the first time the 61-year old Academician has been promised an exit visa.

This time, however, the situation seems more auspicious. The announcement of a visa for Levich and his wife, as well as for 17 other families of Refuseniks, was made by US Senator Edward Kennedy after his recent visit to Moscow, during which he had talks with Mr Brezhnev.

It seems likely that the current promises are designed to alleviate western reaction to the recent wave of 'human-rights' trials in the Soviet Union. Senator Kennedy himself stressed that the granting of the promised visas would improve international relations. Until the Levichs actually arrive in the West, however, the possibility still remains that this promise is nothing more than a diplomatic gambit in the cause of paper detente.

Vera Rich

Science: a means of locating resources

SOMETIMES I fear that we may lose sight of a few realities when we relate science and technology to development. We must be a little guarded, in case too much of the burden for economic development and advancement of countries be placed on the shoulders of science and technology, as a result of which it will be blamed for the failures which will inevitably occur. Science and technology cannot provide, in themselves, the motivation necessary for development. This lies with the political will and in the social drive of the individuals and communities of a country, both in recognising their opportunities and realising them.

In demonstrating that science and technology are nevertheless critical for development, I will limit my comments to economic development. This is not to ignore the social and cultural aspects, or minimize their significance, but because they require a rather different approach. Nor am I concerned, herein, with the current questioning of the value of economic development, as this can too easily generate only social palliatives instead of pragmatic solutions to poverty and inequity, and result in calls for the simple redistribution of wealth at the cost of increasing it overall. It is a separate issue.

The purpose of economic development, is, by and large, the increase in the production of wealth; and the production of wealth takes place by the application to natural resources of man's labour. Under natural resources, I include not only all natural phenomena and associated resources, renewable and non-renewable, transformable and non-transformable, but also the land itself. These are not the product of man. Labour, in this context, is the effort by man which arises from the application of his hands, brain and the tools which his brain has shown him will increase his efficiency. Thus, wealth is the product of man and his resources, which, obvious as it may seem, is often forgotten, as one too frequently reads of natural resources or human resources being regarded as wealth by themselves. The same applies to the scientific and technological capacity of a society. The point is important because loose thinking on it confuses the issues of the production and distribution of wealth and leads to a loss of objectivity in economic development and the assistance which



By
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science and technology can offer it.

The relationship of science and technology to the production of wealth can be seen once it is recognised that a natural resource is no use until it has been found, described and understood. Science, to an increasing degree, is the identifying process in bringing resources into the production of wealth. Technology, however, is related to labour, and the level of technology possessed by labour is the index of its efficiency. Given unlimited natural resources, wealth may be increased without improvement in technology, but with limited natural resources technology must be improved.

This provides a degree of distinction between many developed and developing countries. In general, the former have increasingly to develop their technological capacity, whilst the latter have, for the most part, still to ensure that their natural resources have been identified scientifically and fully realised economically.

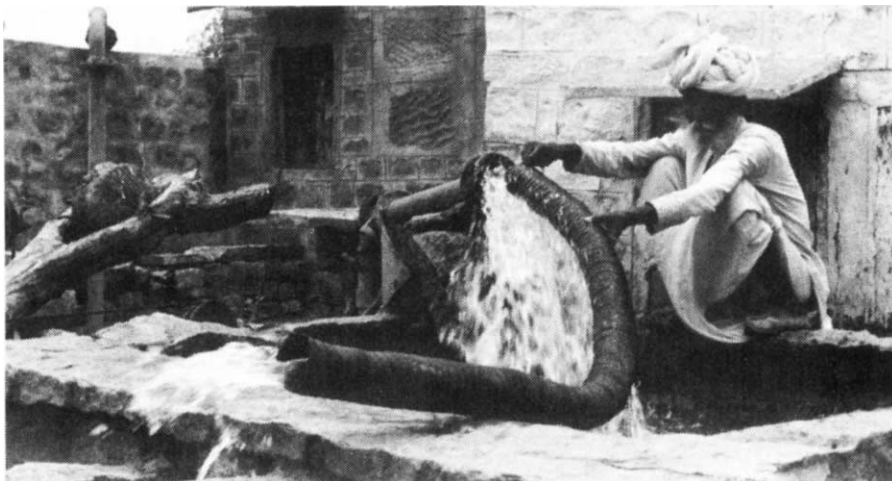
It is important to determine, therefore, if the balance demanded between science and technology is, for any country, the right one in relation to its stage of development and knowledge of its resources. There is a real danger that the cart is being put before the horse: that the developing countries are trying to launch into advanced technologies without an adequate economic justification, when they should still be seeking to upgrade their scientific capacity for its tasks both

of inventory taking and application to renewable resources. Technology is a requirement of circumstances, not an economic panacea. The 'technological gap', as it is called, is as much a reflection of the different needs of countries as it is of restrictive practices, justifiable or otherwise.

The situation must also be seen within the time and circumstances within which it occurs. It has been noticeable that, particularly in countries which in the last decade or so have divested themselves of colonial rule, there has been a decline in the output of the scientific establishments responsible for natural resource inventories and improvement of the yields from renewable resources. This has arisen because of the need of these countries to establish or strengthen their capacity to train their own scientists.

This is a major task, the priority for which has inevitably drawn scientific and technical manpower into the universities, often with better conditions and facilities than exist in the much older applied science establishments. Only now does one see the newly-trained manpower beginning to refill them. It will need more time before able scientists will seek opportunities in industry and only then if its productive capacity and size will sustain viable research and development departments. It is at this stage that the transfer of advanced technologies will really become significant, not only because of the need, but also because there will be an adequate indigenous scientific capacity not only to seize and adapt it, but also, and perhaps more important, to create it.

I think, also, that it is a well to remind ourselves of the basic differences between, and the origins of, science and technology. There seems to be a tendency for administrators, in particular, to regard them as being



Resources: science discovers them, technology applies them

This is a shortened version of a paper presented to a recent meeting on UNCSTD convened by the World Federation of Scientific Workers. The full paper will appear, along with others, in the Report and Documentation of the conference currently being prepared by the Federation.

largely similar in quality and thus to be mutually interchangeable and competitive on the same terms. The development of the so-called applied sciences has further tended, in providing a link between the two, to blur the issue. The result has been, I feel, an over-emphasis on the need for science to justify itself in the same way as does technology, and to have an immediate, direct and finite impact on socio-economic problems. Many scientists have subscribed to this, even if unconsciously, in the search for support for their activities. This too often leads to a shortfall in promises regarding its utility in the eyes of administrators and further strengthens their doubts about the efficacy of science compared to technology.

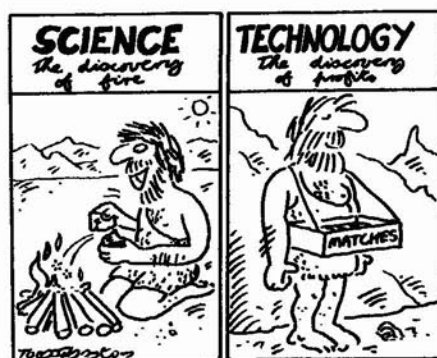
Science, or demonstrable knowledge, is open-ended and has a separate existence. It can be pursued for its own sake, having a cultural value as deeply felt and satisfying as that resulting from the study of art or the humanities, while still serving to relieve man's condition. Technology, in its usually accepted sense, has no existence separate from the material production in which it is involved or for which it was created. Science starts with man's basic curiosity about his environment; whereas technology starts with man's basic economic urge to carry out more easily or profitably a productive activity.

Science, almost by definition, is non-existent until it has been communicated (transferred) to others. Technology has its proof in its products and, therefore, needs not itself to be transferred, thereby conferring rights to its personal ownership and a truly commercial value. Its 'excellence', if I may apply the term, is self-evident. Science, however, needs to be patronised, because its value and excellence is not readily apparent, particularly to administrators. They have to monitor its achievements and excellence, particularly in its more basic aspects, through scientists; and this has, and always will be, an act of faith on their part, which is not a basis for resource allocation they like, particularly since the demand has reached the level it has, today.

Science and technology have been joined in modern times only because much advanced technology has to be science-based; it is now more often manufactured in research and development departments than invented by simple intelligence and manual skill. This, however, does not alter the nature of science and technology, although it does increase the role of science in the production of technology and further emphasises the need for its development.

This brings me to my main point for developing countries, which is that in seeking economic and technical inde-

pendence, particularly in decision-making, they must strengthen their scientific and engineering cadres, not only in their numbers and disciplines, but also in their excellence and problem-solving ability, which latter is the first step to the provision of a genuine indigenous technological capacity. This requires the dynamic teaching of science from the secondary levels of education, through inspired guidance of senior students at the university level, to strictly disciplined research work at the post-graduate level. It means fostering scientific societies, both learned and professional, and the establishment of a stable corps of scientists large enough to be viable and self-critical. It involves increasing considerably the number and quality of



the technical cadres, because most developing countries are characterised by poor ratios of technicians to scientists, which means that any technological improvement or change the latter may prescribe cannot be widely applied.

All this takes a long time and there is no short cut, least of all that based on too early attempts to introduce technologies for which the indigenous technical capacity to handle it is inadequate or absent. We have already seen the mistakes resulting from trying to introduce the applied sciences before the basic sciences are adequately established. To repeat the error in respect of advanced technologies is a greater mistake and can mean an even more extended thralldom, through expensive patents and licenses, to technologies created originally for developed countries.

Moreover, if it is the contention of many developing countries, as it seems to be, that many of these technologies are inappropriate, for whatever reason, technical, economical or social, then it is an even more self-defeating choice as a way to indigenous development. And, it carries with it the threat of limiting the resources for indigenous scientific effort and, thereby, the exploration for, and discovery of, the natural resource potential of a country, which again brings me back to the point I made earlier, namely, that I

believe many of the developing countries need to complete their natural resources inventories before launching into an era of high technology.

I feel that for the realisation of UNCSTD's primary purpose, which is the strengthening of the indigenous technological capacity of countries, that we must think not only of the problems of the choice and transfer of technology but also of the establishment of the right balance between science and technology for development of any country.

Although Unesco's own responsibilities are primarily concerned with the promotion of, and international cooperation in, the natural sciences, whereas other UN agencies have the prime responsibilities for these in the applied sciences, Unesco has increasingly emphasised the important role of natural sciences in relation to the identification and development of the natural resources of developing countries. It is still concerned about the inadequacies both in policy-making in this field and in the creation of effective manpower resources to implement the policy.

In respect of the transfer of technology, Unesco recognises that the problem is much more complicated than that posed solely by the difficulties of gaining access to a particular technology. It involves the creation of a technologically-minded community, not only at the higher levels of specialisation but also at the lower levels of comprehension, where standards and excellence of technical handling are essential for the development of a genuine indigenous capacity which can respond to the needs and opportunities presented to it. Without the development of this basic ability, the transfer of technology alone will remain unfruitful in the long run and tend to separate the communities involved.

For both these reasons, Unesco still stresses the importance of the role of education as one, at least, of the technical prerequisites for strengthening the scientific base of a country and for the successful importation and application of technology. It is, of course, not the only prerequisite. There are social and environmental constraints involved also in the transfer of technology and Unesco has developed programmes in these two fields to examine them and discuss the socio-cultural conditions likely to foster indigenous development processes.

Unesco's activities in this matter of science and technology for development, however, are limited by the availability of funds and the degree of international cooperation it can obtain. We hope that UNCSTD will urge the Member States to be more generous and far-seeing in both these. □

news and views

A massive particle conference

from Frank Close

THE most massive subatomic particle ever discovered was found at Hamburg just one week before a thousand physicists from all over the world gathered in Tokyo for the biennial conference on High Energy Physics. It is the first time that this series of conferences, which began in 1951, had ventured outside of its USA, west-European, east-European cycle. The excitement that has been generated by the discoveries of the last four years has created much cultural interest in the Far East in this most fundamental field of physics, and a delegation from the People's Republic of China was attending such an international gathering for the first time.

The message from the conference was clear. The rapidly accumulating data of the past two years have confirmed our suspicions as to the picture on Nature's jigsaw. The pieces have begun to fall neatly into place and an elegant portrait is emerging.

Unifying the natural forces

The electromagnetic and weak interactions are indeed unified in the "simplest" way, the $SU_2 \times U_1$ model of Glashow, Weinberg and Salam. Briefly, there is much evidence supporting this, and no high energy data that go against it.

To accomplish this unification, Nature has a set of fundamental fermions (quarks and leptons) and has ordered them into left-handed pairs. For leptons we know now that three such pairs (or "generations") exist (*News and Views* 271, 406; 1978): electron and neutrino; muon and a second neutrino; τ and a third neutrino. The existence of the third pair has been firmly established only in the last year. For quarks two generations are well-established. The up and down quarks (found in the proton) have been known for many years, whereas the second generation of strange and charm quarks were established when charmed particles were discovered just before the previous conference in this series (*News*

and *Views* 262, 537; 1976).

Evidence was presented to the conference that a fifth quark (bottom, b) exists. A sixth quark is confidently predicted to complete the third generation of quarks. There is optimism that the advent of PETRA, the new electron-positron colliding beam facility to be opened in Hamburg this month, will provide evidence for this sixth (top, t) quark before the next conference in this series.

The leptons interact by exchanging photons (familiar QED) and also W^+ , W^- and Z^0 "weak gauge bosons". These must be found to really confirm the model. They are predicted to have masses around 70-90 GeV and so we have to be patient for about three years. A session on future accelerators discussed the new machines under plan that may find these objects; and also reviewed the ideas for machines to be built around the world in the longer term and the profound questions that may be asked of them.

The quarks also interact with the above bosons. In addition, quarks carry a quantum number called "colour". New evidence supporting this was presented at the conference: τ decay rates; electron-positron annihilation rates at low energies from Frascati and DCI, Orsay, and the production rates for lepton pairs in proton interactions etc. The quarks can then interact by exchanging coloured gluons. This leads to a theory called "quantum chromodynamics" or QCD (these gluons of QCD are analogues of the photons of QED), and as such provides a theory of the strong (nuclear) interaction.

One consequence is that the scaling phenomenon seen previously in deep inelastic scattering of leptons on proton targets should be violated in a systematic way. In 1976 qualitative support for this prediction was known. It appears that quantitative support is at last emerging now that the older electron and muon scattering data are being supplemented by neutrino interaction data at Fermi Lab and, in

particular, at the CERN SPS in Geneva. This facility became operational in 1976 and the high precision data that it has provided have been instrumental in establishing the weak-electromagnetic unification and are now testing the QCD theory of strong interactions.

Although the emerging data in lepton and in hadron interactions are all consistent with QCD, no conclusive tests that would confirm or deny the theory are yet available. Theorists are still uncovering some of the rich subtleties in the theory and there was much discussion as to the understanding we have of the theory and what we must do to really test it in a severe way.

Heavy leptons

At the 1976 Tbilisi conference the existence of a heavy lepton (τ) was controversial. In the two years since then its existence has been established beyond any doubt by a series of experiments at Stanford (SPEAR) and Hamburg (DORIS). These have made precise measurements of its properties; e.g. its mass is known to be within 0.5% of 1785 MeV (almost the mass of the deuteron!).

All the data suggest that the τ decays by weak interactions producing a (massless?) neutrino and that parity is violated in the process. It indeed appears that just as the electron and a neutrino (ν_e) are brothers in weak interactions (e.g. the radioactive β -decay of the neutron into proton, electron and ν_e), and that Nature repeated itself with a second pair (muon and its neutrino ν_μ), so now we have clear evidence that there is a third generation of leptons: the τ and its neutrino.

Each of these generations violates parity in weak interactions in the same way. It seems that each generation is identical but for two things: the τ is much heavier than the muon (100

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MeV) which is in turn much heavier than the electron (0.51 MeV); just as the muon does not transmute directly into an electron (*Nature* 266, 679; 1977) so the τ does not directly turn into electron or muon.

The data on the τ decay patterns seem to be consistent with the above properties of the τ : decays which should not be seen indeed appear not to be there and decays which should be seen are found. In particular a problem with $\tau \rightarrow \pi + \nu$ (*News and Views* 271, 407; 1978) has now gone away: the decay is seen at the expected rate.

Heavy quarks

Having at last established the existence of three generations of elementary leptons (*News and Views* 271, 407; 1978) attention has turned to the questions of whether there is indeed a third generation of elementary quarks.

In the summer of 1977, the Y (upsilon) particle, 9.4 GeV in mass, was discovered in muon pairs produced by proton-proton collisions at Fermilab (*News and Views* 271, 407; 1978). At the conference it was reported that the Y had now been seen by an independent Fermilab group and also by three groups working at the CERN Intersecting Storage Rings in Geneva.

The real excitement was provided by the groups working at the electron-positron colliding beam machine (DORIS) at Hamburg. It was reported a month ago (*News and Views* 273, 705; 1978) that the Y had been directly produced in electron-positron annihilation, with properties suggesting that it was a bound state of a fifth flavour of quark (bottom) and its antiquark. Just a few days before the conference, the DORIS groups managed to produce a heavier version of this state (Y') with a mass of 10.01 GeV. Measurement of its coupling strength to photons and comparison with the group's data on the lighter Y were discussed and seem to indeed confirm that these are bound states of a bottom quark.

This precise mass determination was communicated to the group at Fermilab who had earlier found the Y in their proton experiment. They do not have the precision of DORIS but their data had shown an enhancement smeared out over 9 to about 10.5 GeV in mass which suggested that two or possibly even three Y -like particles were present. Now that the masses of Y and Y' were known so precisely they were able to look again at their data and found that it indeed requires the existence of a third Y (Y'') with predicted mass of 10.38 GeV. This discovery was telephoned to Tokyo with the recommendation that scientists at DORIS do a high precision search "between 10.379 and 10.381 GeV!".

This may turn out to be one of the first discoveries to be made at PETRA, the new higher energy electron-positron machine opening in Hamburg this month. What is interesting here is that only two Ψ states exist below charm production threshold and it had been predicted that three Y states should exist below bottom production threshold. This verification created much excitement for the PETRA programme since if the top quark exists (completing the third quark generation) then it will be much heavier still with the result that several θ states—bound states of top quark and antiquark—should exist below top production threshold. This gives the prospect of detailed investigation of quark dynamics by studying transitions among these levels of "toponium" analogous to transitions among levels of positronium and hydrogen in atomic physics.

The Hamburg group has studied the decay products of the Y . They have a different angular distribution than the particles produced in the electron-positron annihilation at energies just away from the Y resonance. This is precisely as predicted by QCD. However, it is not yet possible to be entirely sure that the effect is due to QCD as against random filling up of available phase space. This will become clear when the θ ("toponium") is found since at extreme energies the QCD theory predicts angular distributions quite unlike random phase space.

Future prospects

An experiment from Fermilab was reported that shows that particles containing b quarks are not stable, which implies that b quarks decay weakly. Decays of quarks in one generation producing quarks in another generation are familiar (*News and Views* 266, 301; 1977) and are accommodated by the Cabibbo mixing theory. The decay of the b quark implies a new Cabibbo-like mixing involving the third generation with first or second generations. Weinberg stressed that (barring an accident) this necessarily leads to CP violation and hence one of the great puzzles of physics may be about to be solved. Indeed, such a theory of CP violation was postulated by Kobayashi and Maskawa in Japan as long ago as 1972. An important experimental programme will be to study the properties of b decays to see if they are quantitatively in accord with the magnitude of the CP violation which is known to exist.

If these expectations are all verified, then will this be the end of physics? Weinberg highlighted some of the remaining profound questions. Can we unify the strong with the weak and electro-magnetic forces? (some ideas

here were reviewed by Salam). Can we understand the various dimensionless parameters that pervade our universe, like the fine structure constant α , ratios of lepton masses and quark masses; the magnitude of the Cabibbo quark mixing angles and the weak-electromagnetic (Weinberg) mixing angle. The conjecture was that these answers may come as a consequence of a successful grand unification of the natural forces.

Lurking in the background was gravity. Grand unification may take place at energies like 10^{19} GeV (the Planck mass) and the reason for there being distinct forces with different strengths in our current experience may be due to the present coolness of the universe. So how might we achieve this grand unification? Weinberg revealed his ideas—a blank transparency. □

Echinoderm biology

from David Nichols

Two international meetings devoted entirely to echinoderm biology have taken place this year: in March, the Third International Echinoderm Conference was held at the Australian Museum, Sydney, and in July a meeting was organised principally for European workers at Bedford College, London.

At both meetings this year, the proportion of papers offered on the various groups of echinoderms was remarkably similar: notably, about a quarter at each were devoted to starfishes, and a tenth to palaeontological aspects. A major difference in the coverage was that at Sydney about a quarter of the papers were taxonomic, reflecting perhaps the present concentration on this type of work in the Australasian area, while in London only a single paper (by an Australian) was taxonomic.

It would have been all too easy for the Sydney meeting to have been dominated by papers on the coral-devouring Crown-of-Thorns Starfish, but the convener, F. W. E. Rowe (Australian Museum), wisely rationed this subject to three papers. One of these referred to the possible cyclic occurrence of population explosions in this starfish. Elderly fishermen have said that they recall abnormally large numbers of *Acanthaster* being present on the Great Barrier Reef at about the turn of the century, and E. Frankel (University of Sydney) has examined cores taken from the reef talus in several areas to see if he can identify abnormally high concentrations of the disarticulated skeletal ossicles of this starfish among

the broken coral and other material through the cores. He has assumed that the relative occurrence of these ossicles at the various levels in the cores might reflect the relative abundance of the starfish at the time the debris was being abraded from the reef. So far, Frankel has gone back 900 years, and, while not yet recording evidence of a plague 70 or 80 years ago (at the turn of the century), he has recorded higher-than-normal incidences of remains of *Acanthaster* at 110, 185 and 450 years BP.

The conveners of the London conference accepted two papers on *Acanthaster*. First, R. G. F. Ormond (University of York) described a computer model for the population strategy of the starfish, based on data from studies by the Cambridge Coral Starfish Research Group in the Red Sea. The model showed, for instance, that a rise in ambient temperature of a mere 2 °C could produce a population explosion from the 'background' level of 10 to 25 animals per kilometre of reef front to plague proportions of 130 or so. This suggests that *Acanthaster* may lie somewhere near the centre of the r-K strategy spectrum (in MacArthur and Wilson's terminology), and it may vacillate between low and high population levels, as suggested by the periodic plagues shown by Frankel's data.

The second paper on this animal was by J. Vasserot (Station Biologique, Roscoff, France) who suggested that a useful method of control in times of plague might be the use of hypersalinity, which he has found effective against the large European starfish, *Marthasterias*. The method consists of spreading common salt crystals over the aboral surface, and the treatment, which is non-polluting, results in death in a few days. In discussion after this paper, a contributor remarked that this method is already in use by Dutch shellfishermen, who transfer growing mussels from mussel-beds to the holds of their ships, subject them to high salinity, then return them to the beds. This treatment is apparently effective in killing starfish predators adhering to or hiding among the mussels, but does not kill the mussels themselves.

At one time a possible biological control of the Crown-of-Thorns was suggested, which involved seeding the infested reefs with a shrimp, *Hymenocera*, which was known to attack starfishes by piercing the body wall and extracting coelomic fluid. The method was not successful, but it focused attention on the relationship between shrimps and echinoderms. It later began to emerge that such associations are more commonly symbiotic or commensal than predatory. At the Sydney meeting, A. J. Bruce (Director, Heron Island Research Laboratory, Queensland) gave a survey of such associations

in the Indo-West Pacific region, and estimated that about 11% of known shrimps in these waters form some sort of association, mainly with starfishes, sea-urchins and feather-stars. Many use the host merely as a retreat, and emerge on to the substrate to feed; sometimes there may be as many as 50 shrimps on one host; colour patterns in a single species differ according to the host and are often highly cryptic; diurnal colour change has been observed; and some shrimps shave a sea-urchin of some of its spines to make a 'clearing' in which to reside. Little is yet known of these associations, clearly a rich field for future work.

Still with interspecific associations, a highly unexpected spin-off came from some conventional studies of reproductive cycles in the common Australian cushion-star *Patiriella exigua* by D. T. Anderson and C. Lawson-Kerr (University of Sydney), this time between the starfish and a terrestrial caddis-fly. *Philanisus* is a caddis that is known to inhabit the littoral zone in Australia, but until now its intermediate host has been a mystery. During routine sectioning of the gonads of the starfish, these workers observed some egg-strings that clearly did not belong to the starfish, and these were subsequently identified as belonging to the caddis-fly. The female fly was later seen to settle on the dorsal surface of the starfish at low tide and penetrate the dorsal integument in the region of the gonads with a long ovipositor. As yet, the emergence of the young flies has not been observed. This must be one of the very few examples of echinoderms used as intermediate host to a terrestrial organism.

At both meetings there were papers on one of the most controversial aspects of the present-day study of echinoderms, namely, the interpretation of the structure and relationships of a group of fossils variously called carroids, homalozoans or heterosteles. Most echinoderm biologists interpret the unusual fossils in purely echinoderm terms, with a flexible plated body pierced by the usual echinoderm orifices and with various appendages protruding from the body for fixation or feeding. However, in the late 1960s R. P. S. Jefferies (British Museum (Natural History), London) produced a new interpretation of them in mainly chordate terms, referring to them as calcichordates on account of their calcite skeletons. At the London meeting, Jefferies showed how he views both echinoderms and chordates as descended from an ancestor that had settled on the right side of the body (the dextiothetic condition). The calcichordates were seen as the primitive chordate descendants and included forms regarded as primitive cephalochordates, tunicates and vertebrates.

He proposed the term Dextiothetica for the echinoderms and chordates together. The most primitive calcichordates were remarkable in having gill-slits on only the left side of the head, and he pointed out that in the development of the living primitive chordate *Amphioxus* there is a comparable stage with left gill slits only. Jefferies explained this as a straightforward case of Haeckelian recapitulation.

This view has not yet won general acceptance, and at the Sydney meeting G. Philip (University of Sydney) gave a short critique of what he saw as the main points of conflict between the two principal views of these animals. For instance, the 'chordate' school, principally Jefferies, sees the projecting structure of cornutes such as *Cothurnocystis* as a propulsive organ, complete with ossicles ventrally with large muscles above them which were roofed over by immoveable plates; the same structure is seen by those who view the animals as echinoderms as a typical feeding ambulacrum, complete with groove for radial nerve and water vessel, cups for the origin of tube-feet and openable coverplates for protection, very much like an arm of a modern sea-lily or brittle-star. There is clearly a wide divergence of view on the interpretation of these animals, and the hope was expressed in discussion after Philip's paper that the next International Conference should devote a session to this one topic. Much rests on the presumed occurrence of certain types of soft tissue adjacent to the fossilised hard parts that now remain, and perhaps research now underway in Exeter on the nature of the calcite that underlies various kinds of soft tissue will be able to offer additional evidence on whether, for instance, large propulsive muscles really did lie along the projecting stalk of carroids, or whether the crystalline structure of the skeleton in that region is more consistent with other interpretations.

There may now be a break-through in the study of the echinoderm nervous system. Hitherto, workers have been hampered by the difficulty in recording either intra or extra-cellular potentials from individual nerves, because of their close packing and mostly small size. Now, however, it looks as though recording is possible, at least in those echinoderms which have recently been shown to possess giant fibres. An American worker, P. H. Brehm, has succeeded in recording extracellularly from a single nerve axon, using refined suction electrodes on the giant fibres in the radial nerve cord of a brittle-star. J. L. S. Cobb (Gatty Marine Labora-

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tory, St. Andrews) reported to the London meeting that he has repeated Brehm's technique, and has recorded the potential along over 70 cm of a single brittle-star nerve upon photic stimulation. The way may now be open for much further progress in the study of the neurological basis of observed echinoderm behaviour which has until now proved so difficult.

Research effort in echinoderm biology is increasing. An *Echinoderm Newsletter* produced by D. L. Pawson

and M. E. Downey (US National Museum, Smithsonian Institution, Washington) is mailed to over 700 workers, and the three International Conferences have generated a surprisingly large response. At the two meetings this year there was talk of establishing a learned society devoted entirely to echinoderm biology, and of seeking support for a journal. Maybe this reflects a growing awareness that many marine habitats are dominated by this group of macroinvertebrates. □

tends to have an exciton gas without drops, and no drops form above a critical temperature. Other highly interesting recombination effects in the e-h plasma include the possibility of populating the direct minimum in Ge by a band-band Auger effect (Klingenstein and Schweitzer, Stuttgart Univ.).

The weaker concentration dependence of the Auger effect in degenerate ($\sim np$) compared with non-degenerate ($\sim n^{7/3}p$) materials is relevant when setting up the steady state equations for droplets. A stronger dependence even in the degenerate material can again be obtained if k-conservation is neglected or because of higher order effects ($n^{7/3}p$) as has been shown by Haug (*Solid-State Commun.* **22**, 537, 1977).

When we come to bound excitons, it was found some years ago that radiative decay dominates for direct gaps. In an interesting report it was suggested that for small direct gaps the Auger recombination dominates over the radiative mechanism. As the gap is made smaller the Auger effect comes more easily and the radiative transition is less easy. This can be studied in $Hg_{1-x}Cd_xTe$ whose energy gap E_g can be changed by varying x . The two mechanisms are equally important for $E_g \sim 0.5$ eV in this case (Osbourne *et al.*, Caltech, Pasadena). The temperature dependence of the Auger effect gave $\sigma_A \propto n_{ex}^{-N/3}$ instead of n_{ex}^{-2} , where n_{ex} is the extrinsic carrier concentration (Gerhardtts, Berlin and Dornhaus & Nimtz, Cologne). The luminescent decay kinetics for excitons bound to isoelectronic traps in the GaP (the light-emitting diode material) was discussed by Sternheim and Cohen (Haifa).

Multiphonon processes are another field of current interest. The configuration coordinate diagram for ground and excited states of a centre show that (for broadened vibrational energies) one can have at one and the same energy a low-phonon excited state and a many-phonon ground state. The multiphonon transition can be visualised in this way. Indeed the energy given up in this type of recombination (and also in some other mechanisms) can pass to a defect and enable it to diffuse or dissociate (Kimerling, Bell Labs.). One application of this idea (Hutchinson, Birmingham) is that a dislocation emits vacancies into the surrounding lattice as the recombination energy is dissipated, thus increasing trapping defects and causing degradation in devices such as lasers. If this were a

Recombination in semiconductors

from P. T. Landsberg

RECOMBINATION processes in semiconductors have become increasingly important since the invention of the transistor. In semiconductor devices switching and storage times are governed by the recombination lifetimes of excess carriers, as is the photovoltage generated in solar cells, and in light-emitting diodes it is the ratio of radiative to total life times that plays a crucial role. Fundamental work in the field of recombination was the subject of a meeting on Recombination in Semiconductors which took place in Southampton at the end of August. Sir Neville Mott gave the opening address and his support and interest was widely appreciated by the participants.

Many of the semiconductor mechanisms—certainly radiative and Auger recombination—are of interest in plasma physics. This includes the cascading of a non-equilibrium electron down a ladder of states with the emission of a boson at each step. In plasmas and gaseous nebulae for example the boson is a photon and we then have a radiative cascade. Another recent development in this field is the hybrid process of a phonon cascade and multi-phonon process for the last step, when the electron reaches the ground state of a deep centre from its first excited state (N. F. Mott, Cambridge).

What other bosons are there? Should we not also expect in due course to be working on a plasmon-cascade, as narrow gap semiconductors become the subject of more accurate experiments? Although this process does not appear to have been considered before, electron-hole recombination can be greatly increased if a narrow energy gap can be tuned to the plasmon or optical phonon energy. Photoconductivity measurements on semimetallic $Hg_{1-x}Cd_xTe$ alloys reported by Dornhaus and Nimtz (Cologne) have

now confirmed this view. Of course for far infra-red devices it is desirable for good detectivities to find ways of lengthening life times rather than shortening them; coherent plasmon emission is, however, brought closer.

The analogy between semiconductors and plasmas is driven home by low temperature photoexcitation. The electron-hole pairs may then be so tightly packed that there is not enough space for exciton orbits of 100 Å or so, and one then has an electron-hole plasma or liquid. Just as there are droplets formed in the transition between water and water vapour at high humidity, so one has in this case the possibility of drops of electron-hole liquid in an exciton gas. The drops can be made to coalesce by the application of strain fields and so can reach radii from 50 μm to 7000 μm . In a dynamic equilibrium excitons condense on the drops and split up into electrons and holes so as to replenish the pairs lost by recombination within the drops. Studies of these effects were reported by Kelso and Furneaux (Univ. of Calif., Berkeley) who suggested the importance of a constant (i.e. independent of the pair density) term in the drop recombination rate. The experiments can be done for example by studying the decay of luminescence after laser cut-off. While Auger recombination dominates in unstressed drops in Ge, it may be that impurity recombination gives rise to the constant term and dominates in the stressed case. The radiative recombination from the droplets may be expected to be about 8 Mev wide by considering the separation between the quasi-Fermi levels.

Using appropriate reduced variables yields phase diagrams for electron-hole drops which are remarkably similar to those found many years ago for a number of gases. For lower excitation one

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key degradation process, one would try to use dislocation-free material in these semiconductor devices. In solar cells the lattice perfection is of importance (Kazmerski, Solar Energy Research Institute, Golden), as is recombination as a limit on efficiency (Klimpke and Landsberg, Southampton).

Just as it is desirable to design experiments which make the energetic Auger electron visible in some sense when the Auger effect is believed to be important, so also in phonon transitions it is useful to have as direct a way as possible to find evidence for the emitted phonons. This was recently shown to be possible by experiments on GaAs using a superconducting bolometer consisting of a granular Al film (Narayanamurti *et al.*, *Phys. Rev. Lett.* **40**, 63; 1978). They identified phonon emission and explained the fraction of longitudinal and transverse phonons in terms of selection rules. This work has now revealed an anisotropy in phonon generation when observations are made along different crystallographic directions, using n-GaAs epitaxial layers and also p-n junctions. There is a pleasing correlation with light output of dislocated p-n junctions suggesting that this process is an important mode of non-radiative recombination.

It is known that in GaP grown by liquid phase epitaxy dislocations limit the lifetime and hence the radiative output of green light-emitting diodes. Dimitradis *et al.* (UMIST, Manchester) showed how the lifetime increases in GaP as one leaves the neighbourhood of a single dislocation line, and how it approaches the bulk dislocation-free value at the distance of one diffusion length.

The radiative transitions between Landau levels in n-InSb observed in 1972 have now been studied further to find that the lifetime of excess carriers in the first Landau level is inversely proportional to the concentration in this level (Müller & Gornick, Vienna and Bridges & Chang, Bell Labs). Short times of $\sim 10^{-10}$ s are found for concentrations of the order of 10^{18} cm $^{-3}$. A new but expected effect which was reported by Street *et al.* (Xerox, Palo Alto) is the magnetic field enhancement of luminescence in amorphous silicon. It is related to the anomalous photoconductivity and one wonders how general this effect is, since a magnetic field can cause an increase in lifetime by a factor of order 20 at 4 K in Hg $_{1-x}$ Cd $_x$ Te.

Finally, a new technique of recombination centre photocurrent analysis suitable for inhomogeneous distributions of recombination centres was proposed by Lang & Henry of Bell Laboratories. □

Has Atlantis disappeared again?

from D. H. Tarling

THE popular imagination has been captured by the idea that the destruction of Atlantis, as outlined by Plato from Egyptian sources, was in fact a record of the eruption of the Thera volcano in the Aegean some 3,500 years ago—which destroyed the Minoan civilisation not only on the volcano itself, but also on Crete and the surrounding islands. (S. Marinatos *Antiquity*, **13**, 425; 1939; J. C. Luce *The End of Atlantis* Thames & Hudson, 1969). At the same time, scientists concerned with the area who had previously promoted this image seem to be revising their views and are now proposing only tenuous links between the eruption and the decline of the Minoan thalocracy. The problem is that there seems to be a distinct interval between the two events of the order of 50 years. If such a gap really exists, it seems unlikely that the civilisation was ended either by tsunami destroying their fleets or by volcanic ash-fall destroying their crops. This interval is of critical interest to archaeologists and historians, but the succession of events during the largest volcanic eruption in history—at least four times as powerful as the 1883 Krakatoa eruption—is also of interest to a much wider audience. To discuss these matters of common interest archaeologists and Earth scientists gathered for the 2nd International Thera Congress, sponsored by Peter Nomikos, and held aboard a ship cruising the relevant areas of the Aegean.

The geophysical-geochemical background to the Thera volcanic activity was, naturally, based on plate tectonic models in which an East Mediterranean plate is descending to some 110–140 km beneath the Aegean, from which volcanic magmas are currently being derived (H. Berckhemer, Frankfurt; D. Kiskyras, Athens; B. C. Papazachos & P. D. Conninakis, Thessalonika). Geochemical evidence of major, trace and rare earth distribution were in remarkably good agreement for a model in which water released from the descending plate caused partial melting in the mantle wedge overlying it (I. A. Nicholls, Monash; H. Puchelt, Karlsruhe; R. S. Taylor, Canberra) and the magmas are therefore unrelated to descending slab, except indirectly, and are also virtually free of contamination from the thin, overlying Aegean crust. The Aegean, in fact, seems to have a thin crust that is currently in tension (N. C. Fleming, Godalming)—a surprising observation

when it is considered that most of the surrounding areas are under strong compressive stress. This situation is probably related to convective circulation beneath this marginal basin, although it is important to distinguish major differences between this area and the Pacific margin. Around the Western Pacific, earthquake activity proceeds to much greater depths than in the Aegean and oceanic crust is being subducted, whereas the Eastern Mediterranean crust is generally considered to be exceptionally thick oceanic or thin continental crust.

However, as earthquake activity in the Aegean has only been recorded in detail for something like the past 50 years and only with adequate precision for the past 10 years or so, comparison with the Pacific margin is still needed to evaluate possible relationships between earthquake and volcanic activity. C. Blot (Sollies-Pont) made analogies with Guatemala, in particular, to predict that major earthquakes about 100 km beneath Thera would be followed some 1½ to 2 years later by either an eruption or major shallow earthquake on Thera and that this would be followed, some 3 years later, by strong shallow earthquakes in the area of Crete. Unfortunately the precise relationship between earthquake and volcanic activity is still to be firmly established, and many earthquakes seem to be unrelated, in any direct fashion, to magma migrations. It is difficult therefore to apply such models to explain the apparent time-gap between the archaeological evidence for earthquake activity and subsequent volcanic activity and then further possible earthquake activity.

Surprising unanimity was also expressed by volcanologists attending the Congress. There was, for example, agreement that the volcanic deposits were related to only one cycle of volcanic activity and that this activity, by analogy with Krakatoa 1883, probably took place during a single day (C. J. & D. Vitaliano, Indiana; H. Pichler, Tübingen; W. L. Friedrich, Aarhus; R. W. Decker, Dartmouth). This contrasts with previous interpretations of some ash-falls as being related to up to three distinct eruptions, with possible intervals of a decade or so between each fall. This unanimity reflects increasing understanding of volcanic processes and geochemical evidence for the uniformly increasing basicity of the tuffs as the pumice was generated (C. J. Vitaliano *et al.*, Indiana). It is now clear that the earth-

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quake that destroyed the Minoan city of Akrotiri, on Thera, occurred long enough before the eruption—to allow people to return and attempt some initial clearance (C. Doumas, Thera). The eruption itself clearly started with an ash-fall of ~1–2 m thick over the city and was preceded by a very fine pumice fall, only some 1–2 cm thick, which may have been sufficient warning for those clearing the site. The main pumice, however, seems to have been in the form of a base surge, followed by a chaotic ash-flow stage during which large volcanic bombs continued to be spouted out of the volcano. There were clearly no distinct *nuée ardente* deposits, although some pumice debris on wall frescoes has been used to determine the direction of flow of these glowing dust clouds (P. Hédervari, Budapest). One of the most puzzling observations was that preliminary palaeomagnetic data indicated that the walls of the Minoan city had not even been warmed by the volcanic debris and that both the ash-fall, base-surge and ash-flow deposits seemed to have been emplaced very close to room temperature (D. H. Tarling, Newcastle), thereby indicating mud-flow origin (A. Bond & R. S. J. Sparks, *J. Geol. Soc.*, **132**, 1; 1976) at ambient temperatures. Whatever the origin, there was clear agreement that these deposits were all accumulated in a very short time so that the interval between the Thera eruption and the end of the Cretan thalocracy could not be interpreted in terms of a succession of eruptions spread over, for example, 50 years. There was also surprising agreement on the improbability of the pumice ash, that undoubtedly fell on eastern Crete (F. W. McCoy, Columbia), having any great effect on the harvest for that or subsequent years unless the pumice also included poisonous gases, such as fluorine (D. Vitaliano, Indiana). It was even suggested that the thin ash-fall, at most 5 cm thick, could have been beneficial.

The effects of tsunami (I. Yokoyama, Hokkaido), associated with either the earthquake or volcanic eruptions, received surprisingly little direct attention. In general, most earth scientists were mainly concerned with establishing that any tsunami would not have affected ships at sea and any effects would be restricted to the damage of ships at anchor and harbour installations. There is little evidence for harbours along northern Crete at that time (N. C. Fleming, Godalming)—an observation which received little comment yet must have important implications for the idea of a thalocracy governed from Crete. The only solution seems to be that the catastrophic series of events had such a psychological im-

pact that any religious-based administrative system would have collapsed. Nonetheless, such a disintegration would still be expected in late Minoan IA times and not up to 50 years later. The ^{14}C dating of both short-lived materials (grain, shrubs, burnt bone for example) and long-lived materials has raised more problems than it has solved (P. Betancourt, M. Biddle & E. Ralph, Philadelphia). Unlike most other sites, both types of material show an extraordinary range in values, with 70% of the ages for both types of material more than one standard deviation older than the expected 1500–1400 BC date—an age which is known from the good correlations between the precise Egyptian chronology and Minoan pottery styles on Crete. Although the Congress was only presented with the carbon dates, it seems that the only sensible interpretation is that the region of Akrotiri was completely abnormal for well before the earthquake which was to destroy it. It seems unlikely that the ^{14}C dates have been affected by later contamination as most of the materials were well sealed with the archaeological deposits, so the most likely, but still imponderable, explanation is that the vegetation in the area was accumulating varying degrees of old carbon in its structure whilst growing. The processes by which this occurred are unclear, as any volcanic gas emanations would rapidly mix with the atmosphere and clearly much more detailed study is required to establish how such a situation may arise as it is clear that the normal assumptions on which ^{14}C is based do not seem to be valid here and must raise doubts as to their validity in other situations, until the cause of the internal scatter in the Akrotiri materials can be satisfactorily explained.

The archaeological basis for a difference in age between the Thera eruption and the Minoan decline has changed little. In general, most archaeological and historic studies confirm that there was a real time-gap between the Late Minoan IA pottery motifs found in Thera and those extant in the Late Minoan IB period in Crete at the time of the takeover by the Mycenaeans (S. Hood, Oxford; A. Furumark, Uppsala). The evidence suggests that the takeover was not a deliberate invasion but that a Mycenaean exploratory fleet came across a Minoan Empire that was in complete disarray for unclear reasons.

To some extent, therefore, Earth scientists have provided a somewhat more uniform picture of the sequence of events during the Thera earthquake and eruption, but such models are still likely to be drastically modified if archaeological evidence can be ob-

tained for a revision of the relationship between several earthquake events during the period 1500–1400 BC and the volcanic eruption. It will be surprising, however, if such a major series of tectonic events should not be directly related to the otherwise puzzling, rapid decline of the Minoan civilisation at a similar time. □



A hundred years ago

AMONG the resolutions passed by the International Congress on Weights, Measures, and Coins, at Paris, was the following:—The Congress learns with pleasure the progress of the metric system; it deplores that England, Russia, and the United States have not yet entered into the same path; and it is of opinion that the Governments of those countries should be solicited to give effect as early as possible to an act of progress so eminently useful to science, commerce, and international relations." The British and American members had a separate meeting, and resolved to petition their respective Governments to appoint a mixed commission to consider adoption of the metric system by both countries, and to make all necessary recommendations for the proper legislation to secure the desired end.

I AM able to confirm the accounts given by Mr. Simson in your last number as to the probability of the hearing of insects. When travelling on the River Magdalena, New Granada, in 1861, the mode of which is by a long boat, arched over with bamboo, on which the sailors (bogás) passing from one extremity to the other, propel it with long poles, hugging the river bank, accompanied with wild cries and execrations, I observed on several occasions that these cries suddenly ceased, a dead silence following, and on inquiring the cause they pointed to nests high up in the trees, whispering the word *vispa* (wasp). As the bogás pursue their avocations in a state of semi-nudity, they have the greatest dread of these insects, fearing to speak aloud, as their only alternative if attacked by them is to plunge into the stream, where alligators abound. The wasp is long, slender, and black in colour.

From *Nature* 18, 26 September, 576; 1878.

review article

The tau heavy lepton—a recently discovered elementary particle

Martin L. Perl*

This review recounts the history of the discovery of the tau heavy lepton. Evidence for the tau being a lepton is summarised and the known properties of the tau are discussed.

THERE is now very substantial evidence for the existence of a new elementary particle called the tau (τ). (The name τ is taken from the first letter of the Greek word *τρίτον*. It signifies that the τ is the third charged lepton to be found, the first two being the electron and the muon.) Elementary particles are the simplest forms of matter and through studying them we hope to understand the fundamental nature of matter. As I will show in this article, the properties of the τ are analogous to the properties of the electron (e) and muon (μ). Therefore, the τ has been classified as a member of the lepton particle family (Table 1) even though it is much heavier than the e or μ , having a mass of almost $1,800 \text{ MeV}/c^2$. The discovery of this new lepton has particular significance in the world of elementary particles because the lepton family has so few members, and because the τ is so relatively massive. One purpose of this article is to discuss that significance; the other purposes are to outline the evidence for the existence of the τ and to describe its properties as far as we know them.

The first evidence^{1,2} for the existence of τ leptons was found at the SPEAR electron-positron colliding beams facility of the Stanford Linear Accelerator Center (SLAC). The τ 's were produced through the electron-positron annihilation reaction

$$e^+ + e^- \rightarrow \tau^+ + \tau^- \quad (1)$$

a simple reaction whose theory is well understood. All subsequent studies of the τ at SPEAR and at DORIS, the electron-positron colliding beams facility at the Deutsches Elektronen-Synchrotron (DESY), have used reaction (1). (See refs 3–6 for reviews on τ .) Therefore, in this article, I shall limit my discussion of the production of τ 's or other heavy leptons to this reaction.

The unique position of the leptons in elementary particle physics is illustrated by Table 2 which summarises the differences between the lepton and hadron families. The hadrons are composite particles made up of constituents we call quarks; the hadrons interact through the strong interactions which at high energies can lead to very complicated reactions in which many hadrons are produced; and the hadrons occur in more than 100 different types. (See refs 7, 8 for reviews on quarks.) In stark contrast: the leptons are simple, nondisintegrating particles; they do not partake in the strong interactions; even at high energies the reactions in which the leptons directly play a part only produce a few particles (that is, the lepton vertex in the Feynman diagram of the reaction produces only a few particles); and as I have already noted, there are only a few types of leptons. Therefore, the leptons, in contrast to the hadrons, are truly

elementary particles; and in studying the leptons we are studying directly the simplest forms of matter which we have yet discovered. The quarks may also be truly elementary particles; however, we do not know if they can be isolated in a free state; and at present, we can only study them very indirectly. Until the discovery of the τ one might have thought that the simplicity and truly elementary nature of the leptons were connected with their small mass. The e , the μ and their neutrinos have less mass than the lightest hadron—the π^0 with a mass of $135 \text{ MeV}/c^2$. (The name lepton means 'light one' in Greek.) However, the τ is heavier than many hadrons and its existence once and for all separates the concept of a particle's elementarity from the size of a particle's mass. Indeed, we emphasise this by referring to the τ by the oxymoron 'heavy lepton'.

Lepton conservation and the sequential heavy lepton model

Our search for heavy leptons which led to the discovery of the τ was based on the sequential heavy lepton model⁹. We could have used other models^{10,11} in our search; however, the elegance, symmetry and simplicity of the sequential model appealed to us. To our surprise, the properties of the τ are consistent with its being a sequential heavy lepton. The sequential model is based on extending the concept of lepton conservation to heavy leptons. Lepton conservation is the explanation of why reactions such as

$$\mu^- \not\rightarrow e^- + \gamma \quad (2)$$

$$e^+ + e^- \not\rightarrow \mu^+ + e^- \quad (3)$$

do not occur. Taking reaction (2) as the simplest example; lepton conservation asserts: (1) that the e and the μ each possess a unique property not possessed by the other particle, (2) that those properties cannot be destroyed in a reaction, and hence (3) that a μ cannot change into an e . This concept is codified in a

Table 1 The lepton particle family

Name	Electron	Muon	Tau
Charged lepton	$e^\pm$	$\mu^\pm$	$\tau^\pm$
Charged lepton mass (MeV/c^2)	0.51	105.7	$1,782^{+2}_{-7}$
Charged lepton lifetime (s)	Stable	2.20×10^{-6}	$< 3.5 \times 10^{-12}$
Associated neutrino	$\nu_e, \bar{\nu}_e$	$\nu_\mu, \bar{\nu}_\mu$	$\nu_\tau, \bar{\nu}_\tau$
Associated neutrino mass	$< 60 \text{ eV}/c^2$ (may be 0)	$< 0.57 \text{ MeV}/c^2$ (may be 0)	$< 250 \text{ MeV}/c^2$ (may be 0)

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Table 2 Comparison of leptons and hadrons

Family	Lepton	Hadron
Structure	Simple particle, no structure or constituents found by experiments	Composite particle constituents are quarks
Interacts through strong interactions	No	Yes
Interacts through electromagnetic interaction	Only if charged	Yes, in all cases
Interacts through weak interactions	Yes	Yes
Interacts through gravitational interaction	Yes	Yes
No. of types found so far	6 if ν_τ is unique	>100

more mathematical form in Table 3 where the e^- and its associated neutrino ν_e are assigned the 'electron lepton number' $\eta_e = +1$, and so on. Lepton conservation then states that in any reaction the separate sums of η_e and η_μ on one side of a reaction must equal their respective sums on the other side. For example: reaction (2) does not occur because on the left side $\eta_e = 0$ and $\eta_\mu = 0$ while on the right side $\eta_e = +1$ and $\eta_\mu = -1$. In contrast to reaction (3), the reaction



can occur because η_e and η_μ are zero on both sides. Indeed, reaction (4) is a well known and well studied reaction.

An important consequence of lepton conservation is that decay of the μ to an e occurs not through the simple reaction in equation (1) but through the complicated reactions



It is important to note that the decays in equation (5) take place through the weak interactions. The μ cannot decay through the strong interactions because it is not affected by the strong

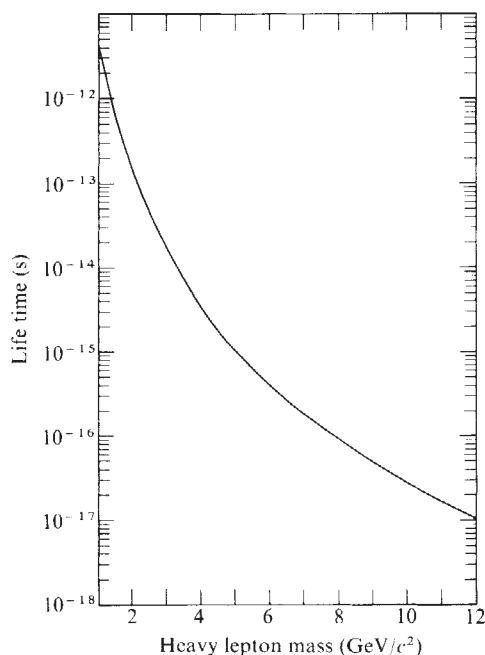


Fig. 1 Theoretical prediction for the lifetime of a sequential heavy lepton with V-A coupling and conventional weak interactions.

interactions; and the μ cannot decay through the electromagnetic interaction because the electromagnetic decay channel, reaction (2), violates lepton conservation. Lepton conservation also dictates how hadrons can decay to leptons. For example, the decays



can and do occur.

Before returning to the sequential heavy lepton model, I should remark that we have no deep understanding of lepton conservation; that is, we do not know how or in what way a μ differs from an e . As an experimenter, I regard lepton conservation simply as an empirical rule whose underlying significance we may one day understand through further experimentation with leptons.

The sequential heavy lepton model⁹ simply assumes that there is a mass sequence of charged leptons and associated neutrinos:

Charged lepton	Associated neutrino
$e^\pm$	$\nu_e, \bar{\nu}_e$
$\mu^\pm$	$\nu_\mu, \bar{\nu}_\mu$
$\tau^\pm$	$\nu_\tau, \bar{\nu}_\tau$
$\vdots$	$\vdots$

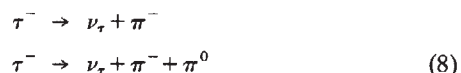
It further assumes that each type of lepton has a unique lepton number which is separately conserved in every reaction. Using this idea and assuming that the mass of the τ (m_τ) is sufficiently large, the τ should decay through the reactions

**Table 3** Values of lepton numbers for various particles

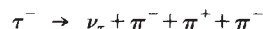
Particle	Electron lepton number, η_e	Muon lepton number, η_μ	Tau lepton number, η_τ
e^-, ν_e	+1	0	0
$e^+, \bar{\nu}_e$	-1	0	0
μ^-, ν_μ	0	+1	0
$\mu^+, \bar{\nu}_\mu$	0	-1	0
τ^-, ν_τ	0	0	+1
$\tau^+, \bar{\nu}_\tau$	0	0	-1
All hadrons	0	0	0

This table assumes the τ is a sequential heavy lepton with its own lepton number η_τ .

These decay channels are analogous to the μ decay channel in reaction (5a): they take place through the weak interactions and they obey lepton conservation. (In the remainder of this article, I shall only write decay reactions for the τ^- , as the τ^+ decay can simply be obtained by reversing the signs of all the charged particles and interchanging neutrinos and antineutrinos.) An exciting consequence of this model is that a sufficiently massive heavy lepton ought to decay to hadrons—just the reverse of reaction (6) in which a hadron decays to leptons. For example, decays such as



and



should occur. These decays would occur through the weak interactions.

The lifetime of a sequential heavy lepton decreases as its mass increases because as the mass of a particle increases it becomes more unstable, and because more decay channels, such as those in equation (8), are available. If one assumes that the sequential

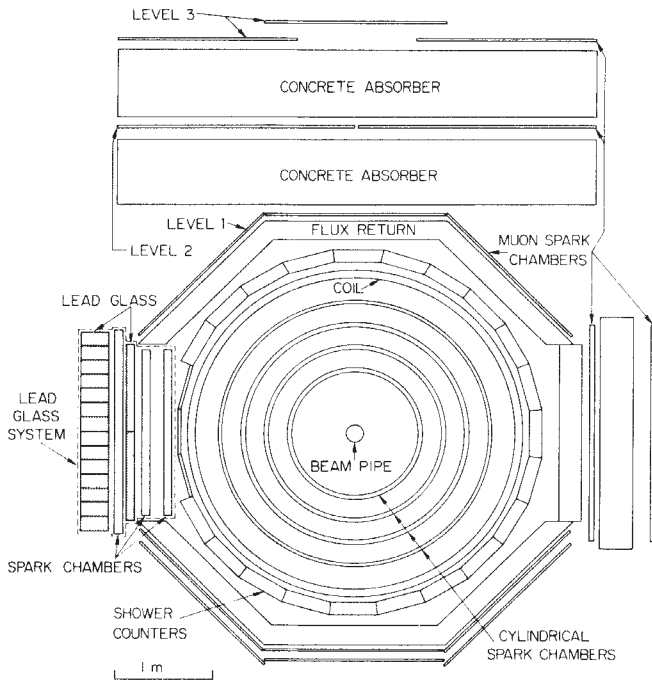


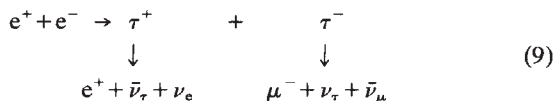
Fig. 2 A cross-sectional view of the SLAC-LBL magnetic detector. The e^- and e^+ beams move along an axis (perpendicular to the paper) inside the beam pipe. The concrete absorber was an addition to the detector used to detect μ -hadron events from τ decays.

heavy lepton has the same type and strength of weak interactions as the μ and e , then its lifetime can be calculated¹¹⁻¹³ (Fig. 1). When we began our search, we already knew that there were no sequential heavy leptons with masses less than $1,000 \text{ MeV}/c^2$. This lower limit had been set by experiments^{14,15} carried out at the ADONE electron-positron colliding beams facility at Frascati, Italy. Therefore, as shown in Fig. 1, the heavy leptons we were seeking would have lifetimes so short that they would decay before reaching our detection apparatus; hence they would have to be detected through their decay products. How this was done is the subject of the next section.

Evidence for the τ particle

$e\mu$ Events. We used the SLAC-LBL magnetic detector at SPEAR for our search (Fig. 2). This detector gathered information on electron-positron annihilation events in which at least two charged particles appeared in the apparatus. We knew that the vast majority of these events had nothing to do with heavy leptons. However, we searched through the events for the events in which only two particles were observed: one an electron and the other a muon, the two particles having opposite electric charge.

These $e\mu$ events could come from the reaction and decay sequence



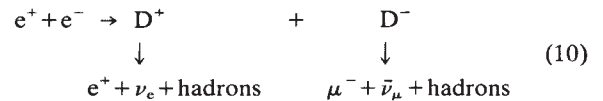
as neutrinos pass unobserved through the detector (Fig. 3). This is a strong signature for heavy lepton production because lepton conservation forbids the production of only an e and μ in the final state; and there are very few reactions in which only an e , a μ and neutrinos are produced. Furthermore, reaction (9) is distinctive because there is on the average a large amount of energy carried off by the neutrinos and hence unobserved.

In the winter of 1974-75 we began to find $^{1,2} e\mu$ events when the total energy, E_{total} , of the e^+ and e^- beams at SPEAR was above $\sim 4,000 \text{ MeV}$. Because in the reaction $e^+ + e^- \rightarrow \tau^+ + \tau^-$ two τ 's must be produced, this meant that the mass of the τ had to be less than $2,000 \text{ MeV}/c^2$. We were also able to place a

lower limit of about $1,600 \text{ MeV}/c^2$ on m_τ by studying the momentum distribution of the e and μ in reaction (9). By the summer of 1976, we were able to show¹⁶ that all the properties of the $105 e\mu$ events we had by then accumulated were consistent with these events being from heavy lepton production.

Figure 4 illustrates one of the consistency tests. The momentum spectrum of the e or the μ produced in reaction (9) through the three-body decays of the τ 's, reaction (7), is described by the solid curve in Fig. 4a. If the τ decayed differently, say by a two-body decay $\tau \rightarrow e + \nu$ or $\tau \rightarrow \mu + \nu$, then the dashed curve would result. Figure 4b shows our measurement¹⁷ of the e and μ spectrum. We could not detect the slow e 's or μ 's required for the low momentum, ascending part of the solid curve in Fig. 4a; however, our measurement was clearly consistent with the high momentum part of that curve. And our measurement is clearly inconsistent with the two-body decay of the τ . A later but more complete measurement of the e momentum spectrum from $e\mu$ events is shown in Fig. 4c. This was obtained by Burmeister *et al.*¹⁸ using the PLUTO detector at DESY.

Of course, the existence of a heavy lepton is not demonstrated simply by showing that $e\mu$ events have properties consistent with those of a lepton. One must also show that there is no other source of the $e\mu$ events. One alternative source which was often suggested was the production and decay of a pair of charmed mesons.



If the final hadrons were not detected, then reaction (10) might resemble reaction (9). My colleague, Gary J. Feldman, was able to show¹⁶ that the majority of our $e\mu$ events did not have undetected hadrons accompanying them.

A very clear proof that $e\mu$ events did have accompanying hadrons, detected or not detected, was produced by the group using the PLUTO detector. They showed¹⁸ that in a data sample which contained 23 $e\mu$ events there were no events of the type $e\mu + \text{hadrons}$.

There was another important result of demonstrating that $e\mu$ events did not have accompanying hadrons. It showed that the reaction



and not the reaction



was the source of $e\mu$ events. We do not expect reaction (12) to

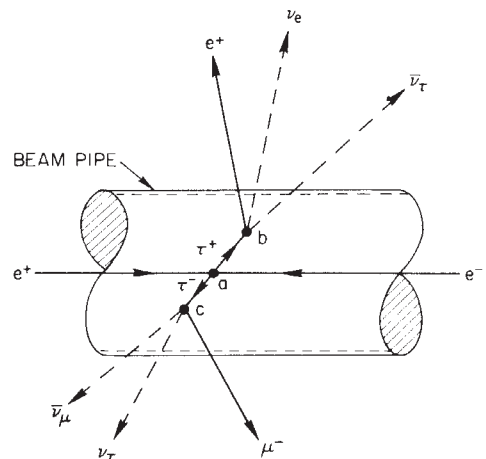
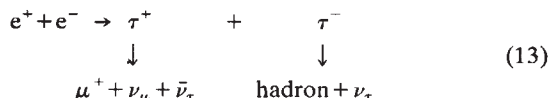


Fig. 3 A schematic diagram of how $e\mu$ events are produced. The τ 's are produced at point (a) where the e^+ and e^- beams collide. They decay close to point (a) at points (b) and (c) while still inside the beam pipe (see Fig. 2). Therefore only the decay products reach the detector. However, the neutrinos, indicated by dashed lines, cannot be observed in the detector; only the e and μ are observed.

be a source of heavy leptons, and the absence of reaction (12) was an additional proof that the τ was a lepton.

μ -Hadron events. If we accepted the $e\mu$ events as coming from the production and decay of a pair of τ heavy leptons, then the sequential heavy lepton model and the mass of the τ being greater than $1,600 \text{ MeV}/c^2$ would require the existence of other rather strange types of events. Thus the reaction and decay sequence



should occur, where the $\tau^- \rightarrow \text{hadrons} + \nu_\tau$ decay is one of the types in equation (8). These events are called μ -hadron events. We were particularly interested in the μ -one-charged-hadron events in which there was just one charged hadron, because such events were unlikely to come from other sources such as charmed meson production. In 1976 a small sample of μ -one-charged-hadron events was found at SPEAR by Cavilla-Sforza *et al.*¹⁶. Later in 1976, after having added an improved muon detection system to the SLAC-LBL magnetic detector (Fig. 2), we were able to report the finding of a large sample of μ -one-charged-hadron events²¹. And physicists using the PLUTO detector at DORIS also found²² such events. As with the $e\mu$ events, all the properties of these μ -hadron events were consistent with the τ being a heavy lepton.

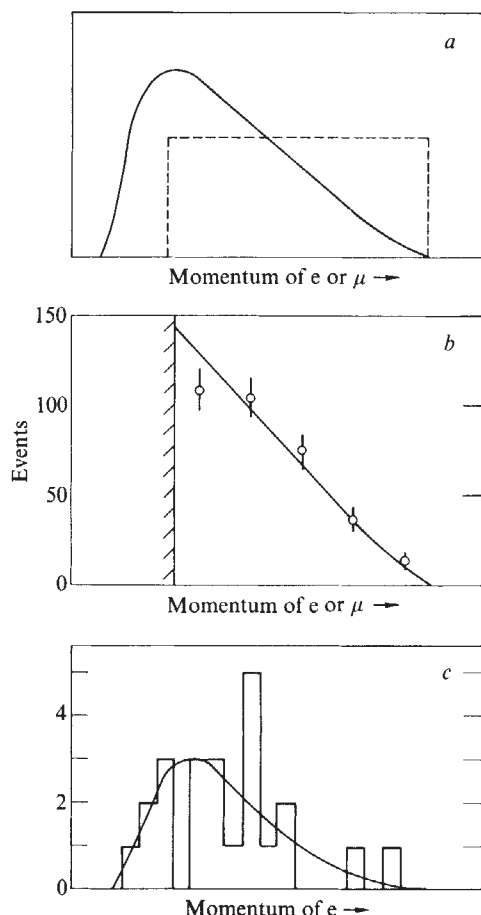
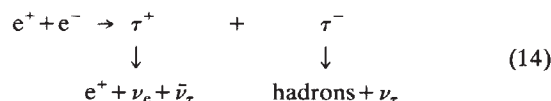


Fig. 4 *a*, The solid curve is the theoretical momentum spectrum of the e or μ coming from the three-body decay $\tau \rightarrow e(\text{or } \mu) + \nu + \bar{\nu}$ of a moving τ . The dashed line is the theoretical momentum spectrum of the e or μ from a two-body decay $\tau \rightarrow e(\text{or } \mu) + \nu$. The comparison of the three-body decay curve with the data is made in (b) for the SLAC-LBL magnetic detector results (ref. 17) and in (c) for the PLUTO detector results (ref. 18). The curves in (b) and (c) are corrected for apparatus acceptance and the spread in E_{total} ; hence they differ slightly from the curve in (a). In (b) the apparatus could not identify e 's or μ 's in the low momentum region marked.

e -Hadron events. The symmetry of the sequential heavy lepton model with respect to τ decays to μ 's and e 's, equation (7), requires that e -hadron events as well as μ -hadron events exist. To find such events it was necessary to have a detector which could precisely separate electrons from hadrons. The DASP detector²³ at DORIS was the first e^+e^- colliding beams detector to have that ability. As shown in Fig. 5, this separation is obtained by using Cerenkov counters. In 1977 the group using DASP was able to find²⁴ the e -one-charged-hadron events expected from the reaction



and more recently physicists using a very large solid angle Cerenkov detector at SPEAR, called DELCO²⁵, have found a very large sample of e -one-charged-hadron events. With the finding of these events, and the determination that their properties were consistent with heavy leptons as their source, the discovery stage of the work on the τ lepton ended. We are now in the stage of detailed study of the τ and measurement of its properties. This is the subject of the next section.

Properties of the τ

Mass of the τ . The most recent measurement of the mass of the τ carried out by the DELCO collaboration²⁶ yields

$$m_\tau = 1,782^{+2}_{-7} \text{ MeV}/c^2 \quad (15)$$

This value was obtained by measuring the cross section, σ_{eh} , for the production of e -one-charged-hadron events as a function of the energy E_{total} . If the τ is a lepton, σ_{eh} should obey the equation

$$\sigma_{\text{eh}} = P_{\text{eh}} \frac{4\pi\alpha^2}{3E_{\text{total}}^2} \left[\frac{\beta(3-\beta^2)}{2} \right] \quad (16a)$$

Here P_{eh} is the probability that a τ pair decays to an e -one-charged-hadron event; α is the fine structure constant; and $\beta = v/c$ where v is the velocity of the τ and c is the velocity of light. It is conventional in e^+e^- annihilation physics to remove the E_{total}^2 term in equation (16a) by defining the ratio

$$R_{\text{eh}} = \frac{\sigma_{\text{eh}}}{\sigma_{e^+e^- \rightarrow \mu^+\mu^-}} = P_{\text{eh}} \left[\frac{\beta(3-\beta^2)}{2} \right] \quad (16b)$$

Here

$$\sigma_{e^+e^- \rightarrow \mu^+\mu^-} = \frac{4\pi\alpha^2}{3E_{\text{total}}^2} \quad (16c)$$

is the cross section for reaction (4). In equation (16b) as E_{total} increases, β approaches 1 and R_{eh} approaches a constant, namely P_{eh} . Figure 6a shows²⁶ R_{eh} . The E_{total} where R_{eh} begins to rise above zero is called the threshold energy, E_{thresh} , and m_τ is given by $E_{\text{thresh}}/2$. The behaviour of R_{eh} in Fig. 6a and of $R_{e\mu}$ in Fig. 6b are consistent with equation (16b) and hence consistent with the τ being a lepton. ($R_{e\mu} = \sigma_{e\mu}/\sigma_{e^+e^- \rightarrow \mu^+\mu^-}$ where $\sigma_{e\mu}$ is the cross section for producing $e\mu$ events.) An older value of m_τ obtained by the DASP collaboration²⁴ is

$$m_\tau = 1,807 \pm 20 \text{ MeV}/c^2$$

Mass of the ν_τ . The DELCO collaboration²⁵ gives an upper limit of

$$m_{\nu_\tau} \leq 250 \text{ MeV}/c^2$$

with 90% confidence; m_{ν_τ} could be zero. The masses of the e and μ neutrinos may also be zero (Table 1).

Lifetime of the τ . The PLUTO collaboration finds

$$\tau \text{ lifetime} \leq 3.5 \times 10^{-12} \text{ s}$$

with 95% confidence. The theoretical prediction for this lifetime is $2.5 \times 10^{-13} \text{ s}$.

The coupling of the τ to the ν_τ . The data favour $V-A$ coupling of the τ to the ν_τ . This, of course, is the coupling predicted by conventional weak interaction theory.

Spin of the τ . Measurement of the behaviour of the cross section for $e^+ + e^- \rightarrow \tau^+ + \tau^-$ as a function of E_{total} strongly favours^{3,4,25} the spin of the τ being $1/2$. This has been discussed in detail by Tsai²⁷. The spins of the e , μ and their neutrinos are also $1/2$, hence the τ on the basis of its spin is a conventional lepton, if we can call a heavy lepton conventional.

Decay modes of the τ . Table 4 lists the observed decay modes of the τ and compares the measured fractional decay rates with the theoretical predictions. These predictions are recent calculations of Tsai¹³ based on conventional weak interaction theory and $V-A$ coupling. In all the measured modes the data and the theory agree; this is another reason for believing that the τ is a lepton.

Lepton type of the τ . All measurements on the τ are consistent with its being a sequential heavy lepton with a unique and separately conserved lepton number. It has been shown^{28,29} that the τ^- does not have the lepton number of the μ^+ or μ^- , nor does it have the lepton number of the e^+ (ref. 30). However, it is possible the τ^- could have the lepton number of the e^- —we have not been able to rule out this possibility experimentally. But this leads to an ugly theory in which the decay

$$\tau^- \rightarrow e^- + \gamma$$

which has not been observed, must be prohibited or suppressed by means other than lepton conservation. My prejudice is that the sequential heavy lepton model, which has guided us so well, will in the end be the correct choice.

Future work on the τ . Studies of the properties of the τ are continuing at SPEAR and DORIS and will also be undertaken at the three new, higher energy, electron-positron colliding beams facilities. These new facilities are PEP at SLAC and PETRA at DESY each with a maximum E_{total} of about 36 GeV, and CESR at Cornell with a maximum E_{total} of about 18 GeV. One direction of these new studies will be to learn more about the decay mode of τ . For example, do decay modes such as $\tau^- \rightarrow K^- + \nu_\tau$ exist at the predicted 1% decay rate? Or does the decay mode $\tau^- \rightarrow e^- + e^+ + e^-$, forbidden by the sequential lepton model, but predicted by other models, exist?

At the higher energy e^+e^- facilities, we can test in more detail our present conclusion that the τ is a lepton. We have come to this conclusion by using Occam's razor and asserting that a charged particle is either a lepton or a hadron. But suppose the τ is some very strange new kind of particle which is 90% leptonic and 10% hadronic in nature. We might expect that the 10%

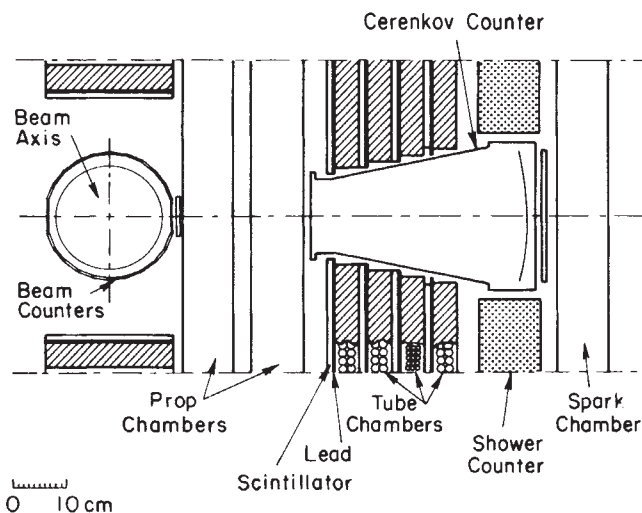


Fig. 5 A section of the DASP detector showing the Cerenkov counter. Redrawn from ref. 23.

hadronic component would show itself at very high energies through the reaction

$$e^+ + e^- \rightarrow \tau^+ + \tau^- + \text{hadrons}$$

If, however, the reaction

$$e^+ + e^- \rightarrow \tau^+ + \tau^-$$

continues to behave at very high energies as it does at the energies now available, then we shall have additional proof that the τ is a pure lepton.

Leptons and quarks

I conclude this article with two remarks on the possible impact of the discovery of the τ on elementary particle physics. First, we have never understood how to calculate the masses of elementary particles. For example, we do not have the least idea why the mass of the electrons is $0.51 \text{ MeV}/c^2$. Perhaps the discovery of the τ will provide a clue as to how to calculate masses because now for the first time we know the masses of three truly elementary particles: the e , μ and τ . (The neutrinos seem to have zero mass which is not much of a help to calculations.)

Second, as noted above, it has become customary to regard the hadrons as composite particles made up of more elementary particles—the quarks. One then regards the quarks and leptons as two different but similar families of truly elementary particles. Furthermore, in some theories the numbers of leptons are equal to the numbers of quarks. For example, before the discovery of the τ there seemed to be four leptons and four quarks:

Leptons: e , μ , ν_e , ν_μ ; Quarks: u , d , s , c

The discovery of the τ upset this equality. However, it may be restored as follows. If the τ is a sequential heavy lepton, its neutrino, ν_τ , is unique and constitutes a sixth lepton. The discovery of the upsilon particle indicates the existence of a fifth quark called b . If we are then willing to believe that a sixth quark, usually called t , also exists, there is again lepton-quark numerical equality:

Leptons: e , μ , τ , ν_e , ν_μ , ν_τ ; Quarks: u , d , s , c , b , t (17)

Many elementary particle physicists would like to see this closed system of twelve elementary particles, with the addition of the intermediate bosons $W^\pm$, Z^0 , constitute the fundamental nature of matter. (I am simplifying here. Most theories also require other particles, such as the gluons, which are supposed to carry the forces between the quarks.) After all, such closed systems of elementary constituents have played crucial parts in physics up to now. We explain the long series of atoms as being made of different combinations of electrons around a nucleus; and we

Table 4 Comparison of predicted and measured fractional decay rates for the τ

Decay mode	Predicted fractional decay rates	Measured fractional decay rates	Groups making measurements
$\tau^- \rightarrow \nu_\tau + e^- + \bar{\nu}_e$ or $\tau^- \rightarrow \nu_\tau + \mu^- + \bar{\nu}_\mu$	16%	16 to 19%	DASP DELCO SLAC-LBL
$\tau^- \rightarrow \nu_\tau + \tau^-$	10%	6 to 11%	DELCO SLAC-LBL PLUTO
$\tau^- \rightarrow \nu_\tau + \rho^-$	23%	$24 \pm 9\%$	DASP
$\tau^- \rightarrow \nu_\tau + \geq 3$ charged hadrons	28%	16 to 32%	DELCO MPP PLUTO SLAC-LBL

Where several measurements have been made, the range of values is given. Numbers are rounded off to the nearest per cent. See refs 3, 4 and 25 for details and references.

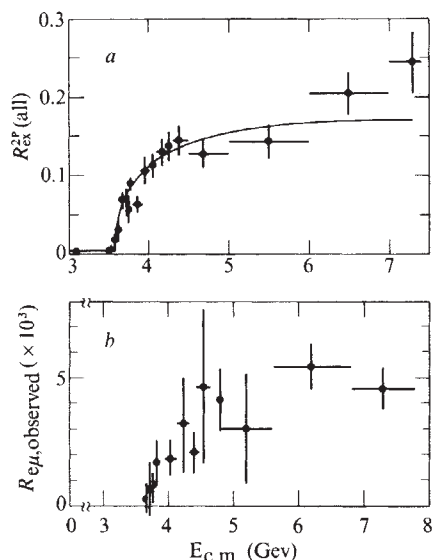


Fig. 6 Behaviour of (a) $R_{ch} = \sigma_{ch}/\sigma_{e^+e^- \rightarrow \mu^+\mu^-}$ from DELCO²⁶; and of (b) $R_{e\mu} = \sigma_{e\mu}/\sigma_{e^+e^- \rightarrow \mu^+\mu^-}$ from the SLAC-LBL magnetic detector collaboration^{3,4}.

explain the hundreds of different kinds of nuclei by different combinations of neutrons and protons. If the system of truly elementary particles is closed as in equation (17), then we will also be able to explain the hundred or more kinds of hadrons as various combinations of quarks. And the lepton will remain a relatively simple and sparse family with the τ being the only heavy lepton.

There is an alternative possibility which intrigues me. Perhaps the τ is the beginning of a long, or even infinite, series of heavy leptons; and perhaps these leptons are truly elementary, as the e , μ and τ seem to be. Then the elementary particle physicist will

face a situation unique in physics: a large number of related entities whose properties cannot be explained in terms of a much smaller number of more fundamental entities. Then our present ways of theorising about elementary particles will not work and we will need some radically new ideas. Of course, this is speculation; and for experimenters, there is an obvious task which precedes this speculation. We must find out whether the τ is the first and last of the heavy leptons, or whether it is only the beginning of a sequence of heavy leptons.

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articles

Recurrent activity in galactic nuclei

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Arguments are presented to support the hypothesis that massive galaxies periodically undergo phases of nuclear activity during their evolution at intervals 10^8 – 10^9 yr. The importance of recurrence to theories of active nuclei is emphasised. In particular, the evidence does not favour the production of massive black holes in galactic nuclei.

THEORETICAL investigations into the problems raised by activity in galactic nuclei have, until recently, concentrated almost exclusively on the hypothesis that nuclear activity (as evidenced by the quasar, Seyfert or radio-galaxy phenomena) is

an essentially singular occurrence in the lifetime of a massive galaxy. The possibility that nuclear activity might be a recurrent phenomenon has been considered, but as yet no compelling reasons for preferring one or other hypothesis have been presented. In this article we assess the observational evidence bearing on this question and outline a theory to account for recurrence. Implications of the establishment of recurrence as a fundamental phenomenon affecting the evolution of galaxies are discussed.

Observational evidence for recurrent activity

As there is no general agreement as to either the nature or the evolution of a galactic nucleus, it is still not possible to determine

unambiguously how any one property of a galaxy relates to nuclear activity, recurrent or otherwise. Nevertheless, a past period of activity might be considered to manifest itself in at least one or more of the following ways in normal galaxies: (1) by the existence of a massive nonstellar remnant²; (2) by a burst of star formation in the nuclear region³; (3) by noncircular motions in the nuclear region⁴; (4) by characteristic radio spectra and structures^{5,6}; and (5) by jets⁷ or unusual arms emerging from nuclei^{8,9}. In some instances, the evidence can testify only to at least one period of activity (for example, (1)), or to recent activity (for example, (5)); but in other cases it is possible in principle to compare the observable timescale of a given signature (τ_0) with the cumulative duration (τ) of the phenomenon inferred from its frequency amongst particular galaxy types. The ratio $R = \tau/\tau_0$ is then a measure of the number of events during a galactic lifetime.

If a burst of massive star formation is triggered by a period of nuclear activity—for example, either by immediate condensation of expelled gas or by passage of shocks—the timescale τ_0 may be estimated from the expected lifetimes of a nuclear H II region or the time required for an initial burst to affect the whole nuclear region, to be $\leq 5 \times 10^7$ yr. The central regions of $\sim 10\%$ of spirals show narrow nuclear emission lines associated with a recent burst of star formation^{10–12}, so in this case $\tau \sim 10^9$ yr, giving $R \geq 20$.

Noncircular motions might in principle have a number of separate causes (such as tidal interaction, or action of a bar), but if it is assumed that those observed are due either directly or indirectly to ejected nuclear material⁴, then observable consequences might survive for $\geq 10^8$ yr (ref. 13). The exact timescale must ultimately depend on the nature and viscosity of the interstellar medium, and for the purpose of the present estimate it will be assumed that $\tau_0 \leq 5 \times 10^8$ yr represents a reasonable upper limit. If the sample of galaxies discussed by Burbidge¹⁴ is indeed unbiased (that is, not selected with nuclear activity specifically in mind), then since about half (excluding Seyferts and barred spirals) display noncircular motions $\tau \sim 5 \times 10^9$ yr, and $R \geq 10$. According to the theory discussed by Clube¹⁵ the very presence of spiral arms is an indicator of recently finished nuclear activity, and if this is accepted then the implied value of R is $R \geq 50$.

The radio luminosities of Seyferts, although not usually comparable with those of powerful radio galaxies, are larger than those of normal spirals by factors of 10 – 10^3 (refs 16–18). It is natural to assume that this difference is a consequence of the nuclear activity, and because relativistic electrons radiating at radio wavelengths have synchrotron half-lives of $\leq 10^8$ yr it might be expected that the effects of their decay could be used as a means of distinguishing single from recurrent activity^{5,19}. However, it is difficult to precisely predict the radio evolution of a post-active galaxy, because the result is sensitive to questions such as the time-dependence of the injection spectrum, diffusion losses, and the magnetic field. It is therefore only possible to conclude firmly that the observations are consistent with the hypothesis of recurrence. For radio sources associated with elliptical galaxies, the structural and spectral evidence favours recurrence more directly, with intervals of $< 10^8$ yr (ref. 19) and independent evidence relating to star formation in these galaxies can also be interpreted as evidence for recurrence³.

Our discussion has been confined to apparently normal post-active galaxies. Another estimate of R may be obtained by considering the Seyfert phenomenon itself. This occurs in $\sim 1\%$ of spirals, giving $\tau \sim 10^8$ yr, while the characteristic timescale associated with the velocities ($\sim 10^3$ km s⁻¹) and sizes (~ 1 kpc) of the disturbed central regions of Seyfert galaxies indicate $\tau_0 \sim 10^6$ yr. Thus $R \sim 100$.

We conclude that the available data are not at variance with recurrent nuclear activity, and estimate a mean interval between active periods $\sim 10^8$ – 10^9 yr. The analysis below applies particularly to Seyfert activity, the kind most likely to be associated with our own Galaxy. The known properties of our Galaxy can thus serve as a comparatively secure basis for calculation.

The physical basis for recurrence: disk formation

Several authors^{1,3,20} have suggested that material lost from stars undergoing normal stellar evolution represents an inevitable supply of gas from which to fuel nuclear activity. If the stars in the galaxy have any residual angular momentum, the gas will in the first instance flow inwards to form a dense gaseous disk, and the idea that formation of a disk is a necessary step in the creation of an active nucleus has frequently been advanced^{21–23}. If this material is ultimately to collapse into the nucleus it is necessary that it lose both energy and angular momentum; and the long timescales associated with angular momentum transport in astronomical systems²¹ suggest that any theory capable of explaining recurrent nuclear activity must use only stellar mass loss from those relatively low angular momentum stars comprising the bulge components of galaxies.

We first show that this source of gas is sufficient to fuel the Seyfert phenomenon. The stellar mass loss rate from a nuclear star cluster of mass M_n and age t may be shown^{24,25} to be

$$\alpha_* M_n \sim 10^{-2} M_n t^{-1} \quad (1)$$

and adopting $t \sim 10^{10}$ yr and $M_n \sim 10^{10} M_\odot$ (values appropriate for our own Galaxy²⁶), this becomes $\alpha_* M_n \sim 10^{-2} M_\odot \text{ yr}^{-1}$. At early epochs ($t \leq 10^9$ yr, say) the mass loss rate is slightly higher than that given by (1), and a more detailed calculation²⁷ shows that the total fractional mass loss during 10^{10} yr is of the order of 10%. The cumulative stellar mass loss in $\sim 10^{10}$ yr in the nuclear region of our Galaxy ($r \leq 1$ kpc) is therefore $\sim 10^9 M_\odot$. The total mass-energy required by the Seyfert phenomenon in this time may be estimated to be $E_{\text{Sy}} \sim 2 \times 10^6 M_\odot c^2$ (assuming a typical luminosity $L \sim 10^{38}$ W and a cumulative duration for the Seyfert phenomenon of the order of 10^8 yr), so even if energy is released at only 'nuclear' efficiencies ($\sim 0.7\%$) the postulated source of gas is still sufficient to fuel Seyfert activity.

An important objection to a model in which nuclear activity is fuelled by stellar mass loss is that supernovae in the nuclear star cluster might heat the gas up to such high temperatures that a steady outflowing galactic wind would occur²⁸. It should be emphasised that this conclusion is only valid provided that heating dominates cooling, and that the system contains no sink²⁹. In the present problem it has been shown³⁰ that steady winds are only favoured in elliptical galaxies at epochs $t \geq 10^9$ yr, with the flow at earlier times being thermally unsteady and directed inwards. The question as to what sort of flow is eventually achieved depends crucially on what happens at time $t \sim 10^9$ yr, since then (when a steady wind first becomes favoured) the flow will initially be inwards. If the disk formed by inflowing gas at early epochs is sufficiently massive and dense it will be able to absorb even hot inflowing gas without heating up, and the appropriate solution to the galactic wind equations will then be one including the presence of a massive disk-like sink. Even if supernovae heat the stellar mass loss with high efficiency the flow can continue inwards, and provided that the sink is strong enough we conclude that outflowing galactic winds do not remove material from the central regions of galaxies.

Physical state, structure and evolution of the disk

To account for the observed nuclear activity it is necessary first that the disk survives as a sink, even when (possibly) hot material is falling on to it; and second that the disk evolves in such a way as to store gas for long periods until a sufficient mass has accumulated that a period of nuclear activity might be produced. To verify that these conditions can indeed be met for a very simple model (more detailed arguments are given elsewhere²⁵), it is assumed that the disk has a surface density

$$\sigma(\tilde{\omega}) = \frac{M_d}{2\pi R_d} \frac{1}{\tilde{\omega}} \cos^{-1}(\tilde{\omega}/R_d) \quad (2)$$

where M_d and R_d are the disk mass and radius respectively. It

may be shown that this density distribution would arise in a model in which the disk is formed out of stellar mass loss from a star cluster with density defined (compare ref. 26) by

$$\rho_*(r) = \frac{M_n}{4\pi R_n} \frac{1}{r^2} \quad (3)$$

and in which it is assumed that the net rotational velocity of the stars at equatorial radius $\tilde{\omega}$ is given by $V_{\text{rot}}(\tilde{\omega}) = \text{constant}$. A model with the same star density but uniform rotation would give a somewhat more centrally condensed disk, but the essentials of the argument do not depend on this detail.

If the r.m.s. gas velocity in the disk is V_s , it may be shown^{31,32} that the disk width $H(\tilde{\omega})$, defined as

$$H(\tilde{\omega}) = \sigma(\tilde{\omega}) / \rho(\tilde{\omega}, 0)$$

is given by

$$H(\tilde{\omega}) = \zeta \frac{V_s}{V_0} \tilde{\omega} \quad (4)$$

where ζ is a numerical factor which, for disks that are gravitationally stable, is of order unity, and V_0 is the (constant) circular velocity in the model: $V_0 = (GM_n/R_n)^{1/2}$. The mid-plane particle density is therefore

$$n(\tilde{\omega}, 0) \sim 3 \times 10^9 \left(\frac{M_d}{10^7 M_\odot} \right) \left(\frac{100 \text{ pc}}{\tilde{\omega}} \right)^2 \times \left(\frac{\cos^{-1}(\tilde{\omega}/R_d)}{\zeta} \right) \left(\frac{1 \text{ km s}^{-1}}{V_s} \right)^2 \text{ m}^{-3} \quad (5)$$

where the mean particle mass has been taken to be $m_H \sim 1.7 \times 10^{-27} \text{ kg}$ and the values $M_n \sim 10^{10} M_\odot$, $R_n \sim 1 \text{ kpc}$ and $R_d \sim 500 \text{ pc}$ have been assumed. The disk is thus very dense, even if V_s should be several tens of km s^{-1} , and it may be expected that cooling, proportional to n^2 , will be efficient. In order to estimate the equilibrium temperature of the disk we consider the balance between heating and cooling processes, as represented by the equation

$$\Gamma = n^2 \Lambda(T)$$

where Λ is the usual cooling function and T is the power input per unit volume from various sources.

Four sources of heating have been considered as of possible importance for the energetics of the disk: (1) heating due to possibly hot infalling material, (2) heating by stars located within the disk, (3) heating by supernovae within the disk, and (4) heating by the effect of stars passing through the disk. On the assumption that stellar mass loss does not lead to further star formation until the disk becomes gravitationally unstable (so that until the point is reached the system contains no massive O-B stars), it may be shown that heating is dominated by hot infalling material. This heat source may be written in the form

$$\Gamma_1 \sim \varepsilon(\tilde{\omega}) \frac{\partial \rho}{\partial t}$$

where ε is the excess energy deposited by unit mass entering the disk and $\partial \rho / \partial t$ is given approximately by $1/H \partial \sigma / \partial t$. The energy carried by unit mass entering the disk consists of a thermal part $\varepsilon_{\text{th}} \sim 3kT/m_H$, and a gravitational part $\varepsilon_{\text{grav}} \sim V_0^2$; so Γ_1 is given approximately by

$$\Gamma_1 \sim \left(\frac{3kT}{m_H} + V_0^2 \right) \frac{1}{H(\tilde{\omega})} \frac{\partial \sigma}{\partial t} \quad (6)$$

By substituting for H and $\partial \sigma / \partial t$ (making use of $(dM_d/dt) = \alpha_* M_n$), and assuming that the infalling material has an initial

temperature $T \sim 2 \times 10^7 \text{ K}$ corresponding to that of a steady supernova-heated flow²⁸, this reduces to

$$\Gamma_1 \sim 7.5 \times 10^{-23} \left(\frac{\alpha_* M_n}{10^{-2} M_\odot \text{ yr}^{-1}} \right) \left(\frac{500 \text{ pc}}{R_d} \right) \times \left(\frac{\cos^{-1}(\tilde{\omega}/R_d)}{\zeta} \right) \left(\frac{1 \text{ km s}^{-1}}{V_s} \right) \left(\frac{100 \text{ pc}}{\tilde{\omega}} \right)^2 \text{ W m}^{-3} \quad (7)$$

Since $\Gamma \sim \Gamma_1$, combining (7) with $n \sim n(\tilde{\omega}, 0)$ gives the value of the cooling function required to just keep the disk at temperature T . That is,

$$\Lambda_{\text{req}} \sim \Gamma_1 / n^2(\tilde{\omega}, 0) \Rightarrow \Lambda_{\text{req}} \sim 10^{-41} \left(\frac{\alpha_* M_n}{10^{-2} M_\odot \text{ yr}^{-1}} \right) \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \times \left(\frac{10^7 M_\odot}{M_d} \right)^2 \left(\frac{\tilde{\omega}}{100 \text{ pc}} \right)^2 \text{ W m}^3 \quad (8)$$

where as before it has been assumed that $M_n \sim 10^{10} M_\odot$, $R_n \sim 1 \text{ kpc}$ and $R_d \sim 500 \text{ pc}$, and factors of order unity have been dropped. Comparing this with the cooling function appropriate for a lightly ionised atomic disk³³ and assuming that $V_s \sim V_{\text{sound}}$ (since strongly supersonic motions are quickly damped), implies the disk will cool—especially in its central regions—to a temperature $\leq 50 \text{ K}$. It is thus concluded that a representative disk of mass $M_d \sim 10^7 M_\odot$ will be cold, and in its central regions will consist of gas mainly in molecular form. The disk therefore does act as a sink, and is able to absorb all the infalling gas due to stellar mass loss without heating up.

If viscous evolution may be neglected during this phase of the disk's evolution (this is verified below), the disk will slowly grow in mass until the onset of gravitational instability. The critical surface density may be shown³¹ to be given approximately by

$$\sigma_{\text{crit}} \sim \frac{\kappa V_s}{\pi G} \quad (9)$$

where κ is the epicyclic frequency, given by $\sqrt{2}(V_0/\tilde{\omega})$ in the present model. Substituting $\sigma(\tilde{\omega})$ into (9) gives the critical mass at which instability first occurs at radius $\tilde{\omega}$:

$$M_{\text{crit}}(\tilde{\omega}) \sim 2\sqrt{2} \left(\frac{V_s}{V_0} \right) \left(\frac{R_d}{\tilde{\omega}} \right) \frac{M_n}{\cos^{-1}(\tilde{\omega}/R_d)} \quad (10)$$

Use of the previously adopted values of M_n , R_n and R_d thus shows that the disk will first fragment close to the origin when its mass reaches

$$M_{\text{crit}} \sim 4 \times 10^7 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) M_\odot \quad (11)$$

The onset of gravitational instability in the disk is expected to lead to a burst of star formation, and if some of the newly-formed stars are sufficiently massive to drive strong turbulence in the disk, the associated viscosity will lead to large central flows of matter. The inwards flux of matter across radius $\tilde{\omega}$ may be shown²¹ to be given approximately by

$$F \sim 2\pi\nu\sigma \quad (12)$$

where ν is the kinematic viscosity. This is given roughly by $\nu \sim \frac{1}{3}\bar{c}l$ where $\bar{c}$ is the mean velocity of a turbulent element and l its mean free path²¹. If l is taken to equal the mean epicyclic amplitude of cloud motions, $l \sim \bar{c}/\kappa$, the kinematic viscosity becomes

$$\nu \sim \frac{1}{3\sqrt{2}} \frac{\bar{c}^2}{V_0} \tilde{\omega} \quad (13)$$

The expected central flux of matter just after the onset of gravitational instability is thus

$$F_{\text{crit}} \sim 2\pi\nu\sigma_{\text{crit}} \sim \frac{2}{3} \frac{\bar{c}^2 V_s}{G} \\ \Rightarrow F_{\text{crit}} \sim 0.06 \left(\frac{\bar{c}}{20 \text{ km s}^{-1}} \right)^2 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) M_{\odot} \text{ yr}^{-1} \quad (14)$$

where $\bar{c} \sim 20 \text{ km s}^{-1}$ has been assumed representative of the mean turbulent velocity in a newly-formed H II region. The flux just before gravitational instability may be estimated from this formula by setting $\bar{c} \sim V_s \sim 1 \text{ km s}^{-1}$, thereby verifying that viscous flows during the quiescent phase of the disk's evolution are indeed small.

If nuclear activity is produced by a period of rapid accretion on to a massive black hole^{2,23,34} energy may be produced with an efficiency, η , approaching 30% (ref. 35). The luminosity generated by such a process is given by

$$L \sim \eta F_{\text{crit}} c^2 \\ \Rightarrow L \sim 10^{38} \left(\frac{\eta}{0.3} \right) \left(\frac{\bar{c}}{20 \text{ km s}^{-1}} \right)^2 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \text{ W} \quad (15)$$

which is very close to the observed luminosities of typical Seyfert galaxies³⁶. From this result we conclude that although other theories of the nature of nuclear activity might be more appropriate (see the discussion), the theory described here is not inconsistent with accretion on to a black hole. Observations of Seyfert galaxies indicate the presence of substantial masses of gas in the nuclear regions^{37,38}, so it is unlikely that normal nuclear activity is able to totally destroy the disk and remove all the gas from the central region. The disk (or parts of it) therefore survives a period of Seyfert activity and the processes described above can be expected to recur, thereby producing a mechanism by which recurrent nuclear activity might be understood.

We note in passing that although conceivable, steady evolution of the disk does not seem likely. For example, it is possible to envisage a steady state in which infall is just balanced by a slow rate of star formation. However, such a situation could only be truly steady if the disk always formed low-mass stars, a possibility that we regard unlikely. The occurrence of even a few massive stars would lead to more³⁹, and eventually turbulent velocities large enough to cause inward fluxes much greater than the infall rate (and sufficient to lead to a period of nuclear activity) would be generated. Massive star formation leads to non-steady evolution. In a different kind of steady state it might be argued (compare ref. 22) that once gravitational instability³¹ has occurred, the heat generated by cloud-cloud collisions and viscous friction could be large enough to maintain a relatively large velocity dispersion in the disk. A steady inward transport at a rate equal to the stellar mass loss rate might then occur, and recurrence (at least on the black hole model) would not necessarily follow. In the present analysis the inward flux immediately after gravitational instability is F_{crit} , implying the viscous heating rate per unit area is given²¹ by

$$D(\bar{\omega}) \sim F_{\text{crit}} V_0^2 / 2\pi\bar{\omega}^2$$

Dividing this by the disk width (equation (4)) for a disk with velocity dispersion characterised by turbulent velocities $\bar{c} \gg V_s$ (where V_s is the velocity dispersion just before gravitational instability) gives the volumetric heating rate due to viscous processes, Γ_{visc} :

$$\Gamma_{\text{visc}} \sim \frac{1}{3\pi\zeta} \frac{V_0^3 \bar{c} V_s}{\bar{\omega}^3 G}$$

that is,

$$\Gamma_{\text{visc}} \sim \frac{5 \times 10^{-24}}{\zeta} \left(\frac{\bar{c}}{10 \text{ km s}^{-1}} \right) \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \left(\frac{100 \text{ pc}}{\bar{\omega}} \right)^3 \text{ W m}^{-3} \quad (16)$$

where the usual values of M_n and R_n have been assumed. Comparing this expression with (7) shows that viscous heating is unimportant to the energy balance of the disk at $\bar{\omega} \geq 10 \text{ pc}$, and even at $\bar{\omega} \sim 1 \text{ pc}$ is only about five times larger than Γ_1 (for $V_s = 1 \text{ km s}^{-1}$ and $\bar{c} = 10 \text{ km s}^{-1}$). The conclusion that the disk will be very cold therefore remains valid, and unless some other energy source intervenes (such as massive star formation) turbulent velocities large enough to drive inward flows of order $\alpha_* M_n \sim 10^{-2} M_{\odot} \text{ yr}^{-1}$ will not be produced. If stars are formed this case reduces to the one above, and steady evolution can only occur if no massive stars form.

Discussion

In the previous sections it has been shown that not only do observations strongly support the idea that nuclear activity in massive galaxies (such as our own) is a recurrent phenomenon, but also this might be rather simply understood by a model in which stellar mass loss leads periodically to nuclear activity via the intermediary of a dense cold (probably molecular) gaseous disk. Arguments based on the rate of viscous transport in the central regions of the disk and the expected evolutionary time-scale of any massive object likely to be formed ($\tau_{\text{evol}} \sim 10^6 \text{ yr}$, say) lead to the conclusion that in the first instance a body with mass in the approximate range 10^4 – $10^6 M_{\odot}$ will be formed (for example, equation (14)). Depending on the detailed evolution of a body of this size, three possibilities may be distinguished.

In the first, it is assumed^{2,40} that any body much more massive than an ordinary star will end its evolution by a period of relativistic collapse, ultimately forming a massive black hole. However, the present theory (based on parameters thought to be typical of our own Galaxy) combined with the observations that the galactic centre does contain large amounts of gas, implies that our Galaxy has undergone recurrent periods of nuclear activity; this is also consistent with the conclusion reached by Lynden-Bell², that the observed numbers of quasars makes it difficult to argue that our Galaxy has never been active. If our Galaxy was active in the distant past, the expected size of the black hole now in its nucleus may be estimated in several ways. First by comparing typical Seyfert or quasar powers with the Eddington limit of a mass M_h ; for $L_{\text{Sy}} \sim 10^{38} \text{ W}$ this gives $M_h \geq 5 \times 10^6 M_{\odot}$, with much larger masses being indicated if our Galaxy has ever been as luminous as a quasar⁴¹. Second, by dividing E_{Sy} by ηc^2 , where η may be estimated³⁵ to be ≤ 0.3 ; this again gives $M_h \geq 5 \times 10^6 M_{\odot}$. Third, by assuming growth at an average rate limited by the stellar mass loss rate²³. With $\alpha_* M_n \sim 10^{-2} M_{\odot} \text{ yr}^{-1}$ this gives $M_h \sim f \times 10^8 M_{\odot}$, where f is the unknown fraction of the mass lost by stars actually transported into the origin; and lastly by assuming growth at a rate limited by the stellar tidal disruption rate^{42–44}, giving $M_h \sim 10^8 M_{\odot}$ provided that the initial hole mass exceeded $\sim 10^6 M_{\odot}$. (Lower mass holes do not grow rapidly in normal galactic nuclei by this process, and the estimate is particularly uncertain as it is possible that the net accretion rate may be much smaller than the tidal disruption rate^{45,46}.) Observationally, current data can be understood without appeal to exotic objects such as black holes^{47,48}, and estimates of the mass within a radius of $\sim 1 \text{ pc}$ ($\sim 4 \times 10^6 M_{\odot}$) are consistent with it being mostly in stellar form^{49,26}. Since observational upper limits to the black hole mass are less (albeit close to) than the theoretical lower limits, we tentatively conclude that black holes are not usually formed in galactic nuclei.

This leaves one of two possibilities. Either the body formed at the centre of the disk explodes disruptively when nuclear burning is initiated^{50,51}, or the body does enter a period of relativistic collapse, but does not form a massive black hole. General relativity has yet to be tested experimentally in the relativistic regime, so this possibility should perhaps not be too lightly dismissed on the ground that it invokes 'new physics'. A detailed discussion of this case is given by Clube¹⁵, who argues that a wide range of independent observations of our Galaxy can be understood most simply on a model in which the Galaxy periodically grows a nucleus whose late evolution does require new physics for its explanation. Here we consider the more conventional

possibility, and note that although the evolution of low-mass supermassive stars as described by Fricke and others does not seem capable of explaining the high luminosities of Seyferts and quasars (approaching in some cases up to $\sim 10^{41}$ W), the nonthermal radiation of a spinar can in some circumstances far exceed the thermal (Eddington-limited) component⁵². It seems likely therefore that the disk will in the first instance form a rapidly rotating disk-like configuration at its centre—resembling a flattened spinar—and although spinar masses are usually assumed to be $\geq 10^8 M_\odot$ (to explain the large energies required by active nuclei for single events), adopting recurrence would lower this requirement considerably. The repeated formation and evolution of low-mass spinars ($M \leq 10^6 M_\odot$) might well explain most nuclear activity, although until the effect on the object of nuclear burning is better understood (that is, whether or not leading to a disruptive nuclear explosion) it is not possible to say with certainty whether 'new physics' is required by the data. It should be noted that stellar mass loss can explain Seyfert energetics even if energy is produced only at nuclear efficiencies.

Thus we have shown here that a wide variety of observational evidence indicates that nuclear activity is a recurrent phenomenon, and that this might be understood on a theory in which activity is caused by gas lost from stars evolving to form an active nucleus via the intermediary of a dense gaseous disk. Following Ozernov and Usov⁵² we conclude that recurrent nuclear activity is best understood by a spinar model, although the present theory indicates that in the first instance only low-mass objects ($M \leq 10^6 M_\odot$) are likely to be formed. Calculation of the late evolution of such a mass depends critically on the effects of the onset of nuclear burning, and our current understanding of this problem does not allow the possibility of gravitational collapse to be distinguished theoretically from that of a disruptive nuclear explosion. From the observational viewpoint the formation of massive compact relativistic objects seems extremely probable⁵³, with one of their most important characteristics being the ejection of both matter and energy⁵⁴.

The hypothesis of recurrence places severe constraints on theories of the origin of nuclear activity. In particular, the apparent lack of a supermassive black hole in our galactic centre argues against the black hole theory, although it should be emphasised that this conclusion does not affect the discussion in the main part of the paper. Because of its potential to discriminate between theories of nuclear activity, further work aimed at distinguishing recurrence from single activity should be given high priority.

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Re-appraisal of lithostratigraphy of Sterkfontein hominid site

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A new lithostratigraphic classification is proposed for the Sterkfontein hominid site. The Sterkfontein Formation is defined in terms of constituent members and beds. The new terminology is correlated with that used previously and, where possible, hominid finds are linked to the newly defined lithostratigraphic units.

THE stratigraphy of the rich site at Sterkfontein, where *Australopithecus africanus* was discovered in 1936, has been investigated several times. Between 1938 and 1952 several

broad descriptive classifications of the deposits were proposed^{1–4}. The first detailed stratigraphic and sedimentological analysis appeared in 1958 (ref. 5); this supplemented by a further generic re-appraisal of portions of the succession⁶, has provided the standard lithostratigraphic subdivision of the deposit until recently. A subsequent geomorphological study of the Sterkfontein cave system⁷ included some deposits. A re-appraisal of the lithostratigraphy of the hominid deposit is necessary because earlier stratigraphic studies did not examine its full vertical extent; the application of contemporary sedimentological measures and stratigraphic classification procedures also seems desirable. In this article I call the hominid-bearing deposit the Sterkfontein Formation.

Location and origin

The Sterkfontein Formation is the largest continuous cave filling at the site ($26^{\circ}01'S$, $27^{\circ}44'E$), and is vertically and laterally continuous through both the Type and Extension sites. It has been exposed both at surface and in the underlying cave system by excavations at elevations of 1,460–1,480 m. At surface the deposit measures approximately 75×15 m. It is exposed on the southern flank of the Sterkfontein hill, the top of which is about 2 m above the highest exposure.

The formation occurs in disconformable juxtaposition with the Malmani Dolomite⁸ of the Transvaal Supergroup, having accumulated within a karst cavity. The sedimentary sequence has been modified periodically by subsidence into underlying cavities formed or enlarged after accumulation of the deposits, by collapse of the cavern walls and roof, by the introduction and dissolution of calcium carbonate cement, and by bevelling of the succession by erosion of the valley flanks of the Bloubaank river (formerly Blaaubank). The Malmani Dolomite here consists of an interbedded succession of dolomite and chert horizons.

The formation of the original solutional receptacle and its subsequent morphological development conform closely with the model of karst development under a spasmodically falling water table proposed for the Transvaal⁹. This initial cavern owed its form to phreatic solution, but was modified by enlargement and collapse in the vadose zone; these modifications were often contemporaneous with the accumulation of the cave filling. The form of the receptacle was controlled by preferential solution along joints and small faults and within locally more soluble horizons in the dolomite, which here dips at approximately 30° NNW. The water table occurs in the underground cave system below the base of the Sterkfontein Formation at an elevation of approximately 1,440 m. Deposits postdating the Sterkfontein Formation are discontinuously present from surface down to this level, and, in places, display complex disconformable relationships with certain of its members.

Although relatively extensive areas of dolomite roof are preserved over parts of the deposit, much of its surface has been bevelled by erosion of the hillside. The inclinations of the lower surfaces of roof segments conform with the local dip of the dolomite bedding planes; a projection of these remnants gives an indication of the maximum elevation of the original roof, which was probably situated some 10 m above the southern part of the deposit. Hence the absolute thickness of the deposit may have exceeded 30 m, less than 70% of which is still preserved.

The original floor of the receptacle varied considerably in elevation; the thickness of the various sedimentary members is very variable and the irregular shape of the depository and obstructing roof pendants produced local variations in the direction of sediment transport. Stratigraphic relationships are consequently complex, and the schematic column in Fig. 1 merely reflects the relative average thicknesses of the various units in accessible areas of the deposit.

Lithostratigraphy

Laboratory analyses were made of large samples (>5 kg) from average vertical separations of <2 m, recovered, as far as possible, from three sampling areas. The procedure involved colour identification using Munsell soil colour charts, removal of CaCO_3 in 10% cold dilute HCl, wet sieving and hydrometer analysis of the residue between 1 and $2,000 \mu\text{m}$ at average mesh size intervals of 0.5ϕ (using sodium hexamataphosphate as a peptising agent), plotting of cumulative textural curves, calculation of Trask indices and statistical parameters using the formulae of Folk and Ward¹⁰, examination of the medium sand residue under a $\times 40$ binocular microscope, and X-ray diffraction analysis of the $-75\text{-}\mu\text{m}$ particle size fraction from selected samples.

The very coarse (>2 mm) fraction of all units was examined visually, and consists chiefly of subangular fragments ranging from pebble to boulder size. The smaller (pebble to cobble) fraction predominates in Members 2 and 6, while large cobbles

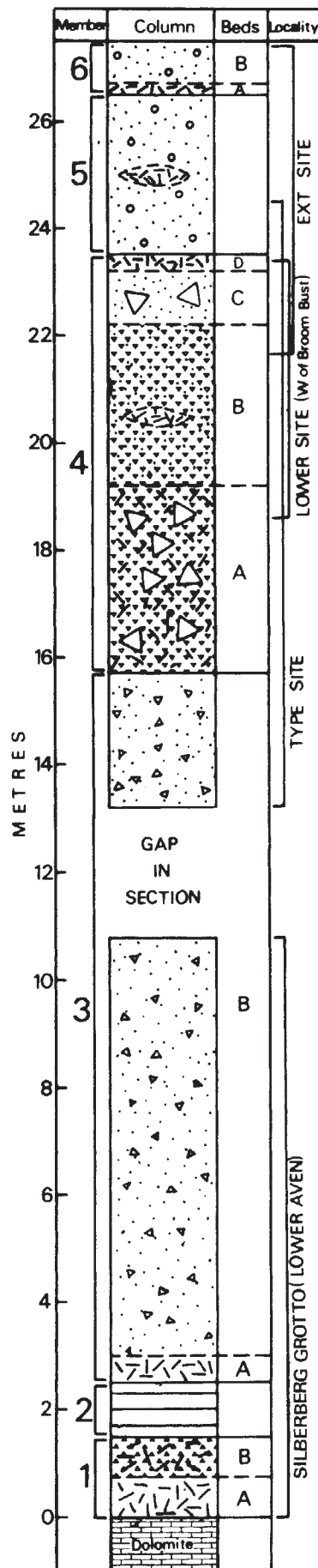


Fig. 1 Stratigraphic column of the Sterkfontein Formation (see text for details)

and boulders are characteristic of bed C of Member 4. In the case of Members 2 and 6 these coarse elements consist almost exclusively of chert, which has sometimes suffered extensive post-depositional snuffbox weathering. In the other units both chert and dolomite are represented; individual fragments, particularly in the larger fractions, are mostly fresh, but may show thin weathering skins on one or two faces, indicating that they accumulated through intracavernous collapses of the roof and projecting ledges. The finer fractions frequently include subrounded chert elements, which are more uniformly and deeply weathered; these represent an extracavernous colluvial component of the deposit. Only in bed B of Member 4 does weathered dolomite contribute significantly to these colluvial elements.

Member 1 is exposed above the weathered dolomite floor in Silberberg Grotto. It consists of 0.5–2.0 m of white banded pale brown (10 YR 7/3) recrystallised meso- and macrocrystalline calcite, locally contaminated with fine clastic sediment. The lower 50%, constituting bed A, has little contamination and no bone. Bed B includes numerous angular chert and dolomite pebbles and cobbles and occasional bone fragments, indicating a small surface opening. The upper contact is wavy and gradational.

Member 2 is exposed in Silberberg Grotto. It consists of 0.5–5.0 m of light brown and red (7.5 YR 6/4–2.5 YR 5/6), mostly centimetre-bedded silty loam (slightly clayey sand-silt) containing numerous broken speleothems, occasional calcite lenses, scattered pebbles and cobbles, and high concentrations of bone near the base. Deposition has occurred around a large stalagmitic boss belonging to Member 1; to the east, bedding planes dip approximately 10° SE, while to the west, the sediments comprise a steep debris cone. CaCO₃ averages 73% and average ϕ mean is 5.20. Sediments are poorly to very poorly sorted¹¹ and highly leptokurtic with pronounced negative skewness. Crystalline minerals (in decreasing abundance) are quartz, pyrophyllite, sericite, goethite and kaolinite. Typical particulate roundness and sphericity are 0.1 and 0.5 respectively, indicating higher angularity than is characteristic of present external colluvial soils. As in all succeeding units, quartz particles predominate; chert is present in very small proportions and dolomite grains are rare. Surface staining is largely absent. Contact is slightly wavy and abrupt.

These features suggest periodic ponding and episodic subaqueous deposition with flushing of some fines into more stable depositories to the east of the boss, and conical gravitational accretion to the west. Sedimentary parameters suggest higher energy conditions near the middle of the bed. Distribution of this and succeeding members, and direction of depositional dip relative to relief in cave floor, indicate the presence of an opening vertically above the eastern end of Silberberg Grotto.

Member 3 consists of beds A and B. Bed A is exposed in Silberberg Grotto and is composed of recrystallised mesocrystalline calcite averaging 0.5 m thick and dipping about 15° SE. Bed B is exposed in Silberberg Grotto and the East Pit of the Type Site (top of bed only). It consists of about 10 m of reddish brown (5 YR 5/4) occasionally speckled red (5 YR 5/8) loam (clayey silty sand) containing scattered angular pebbles, cobbles and boulders and abundant bone fragments. Bedding is not discernible, but calcite lenses occur in lower levels. CaCO₃ averages 76% and average ϕ mean is 5.10. Sediments are very poorly sorted, leptokurtic and very positively skewed. Crystalline minerals are quartz, goethite, sericite, feldspar and kaolinite. Typical roundness and sphericity are 0.3 and 0.5 and compare with outside colluvium. There is occasional pyrolusite staining. Contact is apparently conformable and abrupt. The sedimentology indicates slow, uniform gravitational accretion of external colluvium and, possibly, some aeolian sand through small openings without significant resorting by water, but with occasional collapses of protruding cave ledges.

Member 4 consists of beds A, B, C and D. Bed A is exposed in the Type Site and the Lower Cave. It consists of 1–5 m of

angular pebbles, cobbles and boulders cemented by recrystallised meso- and macrocrystalline calcite, containing occasional small irregular pockets of reddish brown (5 YR 5/4) silty loam (slightly clayey silty sand) and occasional bone fragments. Fine sediments were apparently contemporaneous with the coarse fraction, and are similar to those of bed B, with a ϕ mean of 4.90. Contact is indigitating. This bed indicates a series of major roof collapses in rapid succession, greatly enlarging the cave opening. This is supported by analysis of the ¹³C content of the calcite within succeeding units; a significant increase in ¹³C above this level suggests greatly improved ventilation of the cave¹².

Bed B is exposed in the Type Site, the Lower Cave and lower levels of the Extension Site. It consists of 1.0–5.5 m of predominantly reddish brown (5 YR 4/4 and 5/4), but less frequently yellowish red (5 YR 5/6) and red (2.5 YR 4/6 to 5/8) sandy loam (slightly clayey silty sand) containing abundant pebbles, cobbles and boulders and occasional bone fragments. Crude stratification is present in the very coarse fraction, parallel to the flanks of a debris cone with its apex near the southern margin of the Type Site excavation. Infrequent recrystallised calcite lenses occur on the flanks of the cone. CaCO₃ averages 53% and average ϕ mean is 4.47. Sediments are poorly to very poorly sorted and leptokurtic with a wide range of skewness from negative to positive. Near the cone periphery (for example, in the Lower Cave) sediments are less negatively skewed and less markedly leptokurtic. Crystalline minerals are quartz, goethite, sericite and kaolinite. Typical roundness and sphericity are 0.3 and 0.5, and particles often show pyrolusite staining. Contact is eroded and disconformable.

The characteristics of this bed are consistent with discontinuous redistribution of gravelly colluvium, in admixture with roof collapse debris, down the flanks of a cone of gravitational accretion under the influence of sheetfloods entering through an enlarged cave opening. Higher energy conditions are evident towards the middle of this unit, especially around the periphery of the cone.

Bed C is exposed in several large, isolated pockets in the Type Site and the Lower Cave. It consists of 0.5–2.0 m of yellowish red (5 YR 5/6) or red (2.5 YR 4/8) unbedded sandy loam (slightly clayey silty sand) containing occasional angular cobbles and boulders and localised concentrations of bone. CaCO₃ averages 53% and average ϕ mean is 4.11. Sediments are very poorly sorted and mesokurtic to leptokurtic with marked negative skewness. Crystalline minerals are quartz, sericite, kaolinite and goethite. Typical roundness and sphericity are 0.3 and 0.5, and pyrolusite staining is common. Contact is even and abrupt.

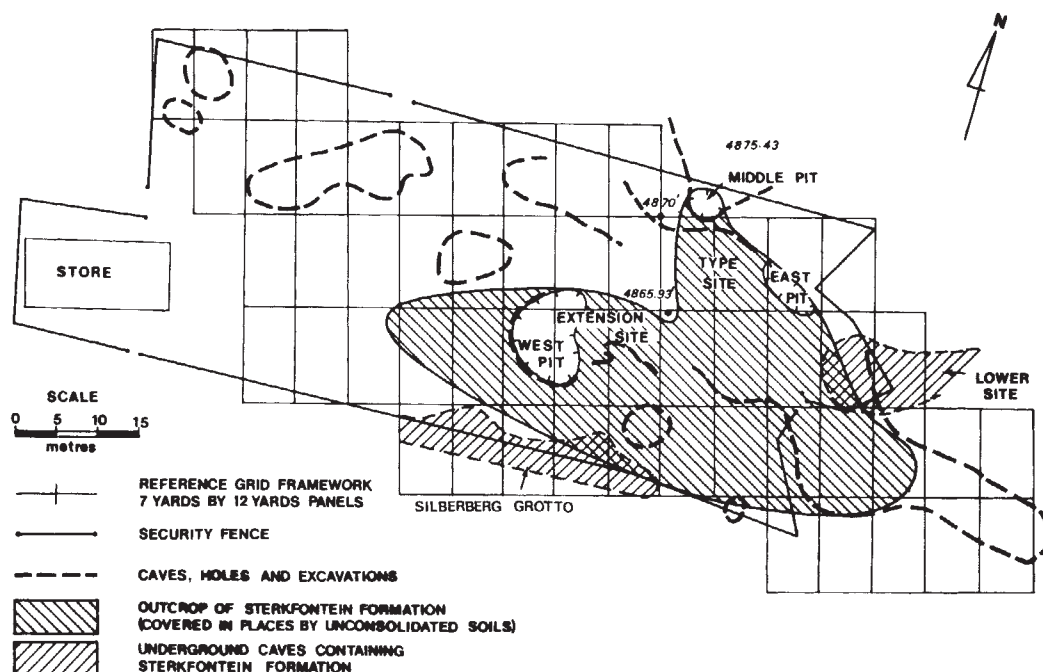
There seems to have been greater dominance of water action than in bed B, with flushing out of fines. The gravelly component of the external colluvium (if present) was not introduced during

Table 1 Comparative terminology of Sterkfontein Formation

Terminology	Previous (Robinson ⁶)	Present	Hominid remains
Upper breccia (Extension Site)		Member 6	—
Middle breccia (Extension Site and uppermost Type Site)		Member 5	StW 53 and stone industry
Lower breccia (Type Site, Lower Cave and base of Extension Site)		Member 4	Bed C StS5*, StW 12, 13, 14 & 17 Bed B StS 17*, 71* The remainder of the specimens of <i>A. africanus</i> were recovered either from bed B or bed C

* Correlation inferred from Broom *et al.*'s²² description of host deposits.

Fig. 2 Locality plan showing the surface extent of the Sterkfontein Formation and the positions of localities mentioned in the text.
(After A. R. Hughes.)



this period, and intracavernous collapses were less frequent. Higher energy conditions prevailed during deposition in the middle of this unit.

Bed D is exposed in the Type Site and the Lower Cave, vertically above pockets of bed C. It consists of discontinuous white to brownish (10 YR 5/3) horizontally laminated, recrystallised meso- to macrocrystalline calcite lenses up to 75 cm thick. These consist of accumulations of thin calcite rafts which occasionally form in pools in contemporary Transvaal caves. Contact is unconformable and eroded; there has been minor subsidence of Member 4 before accumulation of Member 5.

This bed represents a period of greater abundance of water in the cave. The presence of very large collapse blocks incorporated in the calcite cement indicates another episode of roof instability.

Member 5 is exposed at the Extension Site and the western end of the Type Site. It consists of 1–5 m of reddish brown (5 YR 5/4 and occasionally 4/4) sandy loam (slightly clayey silty sand) containing numerous pebbles, cobbles and boulders, mostly of chert and dolomite, but including foreign quartz, quartzite and diabase, which are believed to have been introduced by hominids¹³. Bone is occasionally present, and a few calcite lenses occur near the middle of the member. Crude bedding, mostly conformable with the eroded surface of Member 4, is evident in the very coarse fraction. CaCO_3 averages 54% and average ϕ mean is 3.85. Sediments are very poorly sorted and meso- to leptokurtic with negative skewness. Crystalline minerals are quartz, goethite, feldspar, sericite and kaolinite. Roundness and sphericity are similar to those in Members 3 and 4, but pyrolusite staining is less frequent. Contact is abrupt, eroded and unconformable.

The mode of accumulation was as in bed B of Member 4, but without the introduction of weathered dolomite colluvial gravels. Differences in the range of skewness can be ascribed to the relative positions of exposures within the depositional cone. The configuration of crude stratification confirms the location of the main cave entrance to the south of the Type Site. Evidence of cyclically higher energy conditions is present near the middle of this member.

Member 6 is exposed on the north wall of the West Pit excavation in the Extension Site. It consists of beds A and B. Bed A is composed of up to 25 cm of contaminated and recryst-

allised basal calcite, which is occasionally bone-bearing. Bed B is 0.5–1.0 m of dark reddish brown (5 YR 3/3) sandy loam (clayey silty sand) containing occasional pebbles and cobbles, including foreign stone and some bone. A single sample from bed B had a CaCO_3 content of 47% and a ϕ mean of 5.10. Sediments are very poorly sorted out and highly leptokurtic with positive skewness. Crystalline minerals are quartz, sericite, kaolinite, goethite and feldspar. Roundness and sphericity are 0.5 and 0.7 and a large proportion of particles shows pyrolusite staining.

Bed B is similar to the pebbly colluvial soils which now occur on adjacent areas of the Sterkfontein hill; however, foreign stone is absent from the latter, and sand grains of Member 6 are less angular.

Extracavernous source materials

Comparative analyses of deposits from the vicinity indicate that the -2-mm fraction of the various units was derived overwhelmingly from extracavernous colluvial sources. The contribution of the insoluble residue produced by intracavernous corrosion is chiefly restricted to a few highly angular chert particles.

Several generations of colluvium are evident near Sterkfontein; these range from the pebbly, skeletal mantles in areas where a balance exists between erosion and deposition, to less frequent, deeper, pedogenetically differentiated profiles in relict areas immune from rapid denudation. The -2-mm fractions usually display fairly similar ranges of properties; these seem related rather to a geomorphic environment of episodic incision along the Bloubaank valley and to local depositional conditions than to climate and weathering, the imprint of which should rather be sought in fluctuations in patterns and rate of sedimentation within the protected environments of cave depositories.

The characteristics of the Sterkfontein Formation are most readily explained in terms of the reworking of source materials akin to the present, widespread, thin, pebbly colluvium. This is a dark reddish-brown (5 YR 3/4), sandy loam (slightly clayey silty sand), containing abundant weathered chert pebbles and cobbles, and seldom exceeding 50 cm thick. The ϕ mean averages 4.20. The material is poorly to very poorly sorted and is leptokurtic with a small range of skewness, both negative and

positive. Crystalline minerals are quartz, goethite, kaolinite, sericite and feldspar. Roundness and sphericity are 0.3 and 0.5, and many particles show pyrolusite staining.

Sedimentary facies of the various units of the Sterkfontein Formation can be related to the extracavernous colluvium in terms of a limited suite of transportational and depositional processes. Except in the case of Member 3, where enrichment in fines, possibly due to the presence of an aeolian component, has resulted in pronounced positive skewness, the colluvial source materials have suffered removal of fines to various degrees. This can be attributed to the flushing action of successive sheetfloods through the cave opening, which tended to transport fine elements into more distant depositories within the cavernous dolomite, increasing negative skewness except where further transportation was obstructed (for example, in areas where debris cones impinged on the walls of the cave).

Fine sedimentary fractions are more abundant in the lower members, due to the greater degree of illuviation with increasing distance from the cave opening. An exception is Member 6, the proximity of which to the cave opening would be expected to favour a depletion in fines through flushing and eluvial effects. The relatively high proportion of fines encountered in this member suggests that the colluvial source materials have undergone limited transportation into a restricted depository with enrichment in fine elements.

The extensive hiatuses in clastic deposition within the cave, sometimes associated with calcite accretion, indicate cycles with reduced influx of extracavernous sediments. In some instances such episodes were marked by intra-cavernous scouring and the production of erosional unconformities.

Under the present climatic and pedogenetic regime in the area the very coarse fraction of the colluvium consists exclusively of partially weathered chert fragments derived from the chert-rich dolomite. Material of this type is present in all members, and has sometimes undergone post-depositional weathering. Only in bed B of Member 4 does weathered dolomite form a significant component of the very coarse colluvial fraction. The contemporary occurrence of weathered dolomite in colluvial mantles in South Africa is restricted to arid environments.

Solution cavities and discrimination between members

Mention must be made of the unconsolidated fillings of solution cavities which were formed within the soluble surface of the Sterkfontein Formation, chiefly of Member 5, after the cessation of sedimentation. These attain depths in excess of 5 m and contain material produced by decalcification and *in situ* weathering of the surrounding sediments. A clear transition exists between the overlying recent colluvium and these residual materials. The latter sometimes contain bone. The weathering residue from Member 5, which occurs within these cavities, is sedimentologically similar to its parent material, but somewhat better sorted, indicating enrichment in fines, either through *in situ* weathering and comminution of particles, or through illuviation from the overlying colluvium. Individual particles are also more rounded than those from Member 5, and pyrolusite staining is widespread.

Sedimentological analysis of samples from the various lithostratigraphic units of the Sterkfontein Formation reveals that successive members are distinctive in terms of the standard deviation of at least one of the associated quartile and moment measures.

Comparison with Swartkrans Formation

The stratigraphy of the nearby Swartkrans hominid site has been re-appraised recently^{14,15}, and the Swartkrans Formation has been defined. Comparison of the Sterkfontein and Swartkrans Formations shows that, although similarities exist, there is no obvious lithostratigraphic overlap between their various members and beds. The sediments of the Swartkrans Formation are distinctly more positively skewed and are generally more

poorly sorted than those of the Sterkfontein Formation. While the accretion of Member 1 of the Swartkrans Formation in a gravitative debris cone is analogous to that of Members 4 and 5 of the Sterkfontein Formation, the subsequent large-scale corrosion of this accumulation, and the filling of the voids thus created with younger sediments, followed, in turn, by a second cycle of channel erosion and deposition, is not apparent at Sterkfontein. The preponderance of sediments derived from deep argillic colluvium in both Members 1 and 2 of the Swartkrans Formation¹⁴ likewise has no parallel at Sterkfontein.

The faunal assemblage from the Type Site at Sterkfontein (labelled StS) is overwhelmingly derived from Member 4 of the Sterkfontein Formation; it is very different from that of the Extension Site, and has no equivalent at Swartkrans¹⁶. Previous evaluations of the assemblage from the Sterkfontein Extension Site¹⁷⁻²⁰ have assumed that the entire West Pit assemblage (labelled SE) belonged to one stratigraphic unit. My studies reported here, confirmed independently by C. K. Brain, reveal that some specimens from this assemblage have attached matrix belonging to Members 4 and 6 of the Sterkfontein Formation, even though Member 5 is the most widespread stratigraphic unit exposed in the West Pit. In view of this discovery, previous evaluations of these faunas with respect to chronology will have to be revised. A much needed comparison of the faunas of Member 1 of the Swartkrans Formation and Member 5 of the Sterkfontein Formation awaits a further study of the revised assemblage from the latter unit (to be undertaken shortly by E. S. Vrba).

The fauna from Member 2 of the Swartkrans Formation¹⁵ includes elements from both the main mass of this unit and the later channel fillings. Vrba's¹⁶ earlier and later subdivisions of the SKb bovid fauna can be equated with these two units. Vrba^{16,21} has concluded that the later SKb fauna corresponds to her later Sterkfontein category, which is likely to belong to Member 6 of the Sterkfontein Formation.

These comparative faunal data are not sufficiently precise to permit postulation of time-equivalence between the respective stratigraphic units.

Correlation with previous terminology and hominid finds

It may prove useful for readers familiar with the published stratigraphic terminology of previous workers at Sterkfontein to have a cross-reference to the terminology used here (Table 1). At the same time, an indication is also provided of the relative position of the excavations carried out to date and the known of likely provenance of certain of the hominid remains.

From the above correlation it will be clear that excavations so far have sampled only the uppermost 30% of the stratigraphic thickness of the Sterkfontein Formation. No excavations have been carried out in Members 1-3.

I have not considered biostratigraphic or chronostratigraphic aspects of the Sterkfontein Formation here; these, together with the environmental implications of the data, will be discussed elsewhere.

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Neuronal properties underlying processing of visual information in the barnacle

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Generation of a transient, amplified response to the dimming of light in the visual system of the barnacle involves two synaptic stages. It is accomplished primarily by decrementally conducting neurones that are similar to bipolar cells of the vertebrate retina.

THIS work is concerned with the fate of visual information as it is processed by the first few cells of the simple visual system of the barnacle. The specific problems addressed here have been raised by observations on the vertebrate retina. As information is passed from the receptors to bipolar cells and from them to the retinal ganglion cells, the steady responses that encode light intensity are essentially lost, whereas responses to changes in intensity are amplified. How the synaptic and membrane properties of decrementally conducting retinal cells accomplish this process of differentiation, which enables ganglion cells to perceive changes over a wide intensity range, is not understood (see, for example, refs 1–4). Like their counterparts in the vertebrate retina, the first few cells of the visual pathway of the barnacle are specialised to extract information about changes in intensity.

Voltage changes induced by light in the dendrites of the kphotoreceptor cells of the barnacle median eye spread decrementally along the cells' axons to their terminals in the supra-oesophageal ganglion^{5,6}. Light depolarises the photoreceptors and dimming causes them to repolarise. In cells which we conclude to be second- and third-order, however, dimming causes a transient depolarisation. This depolarisation generates a burst of action potentials in the third-order cells. These impulses are conducted through the circum-oesophageal connectives to the ventral ganglion to influence neurones that mediate the shadow-induced withdrawal of the animal into its shell^{7,8}. The present study is of the synaptic connections and membrane properties of the neurones that process the receptor signal to generate an 'off-response' over a wide range of light intensities.

Identification of second- and third-order ganglion cells

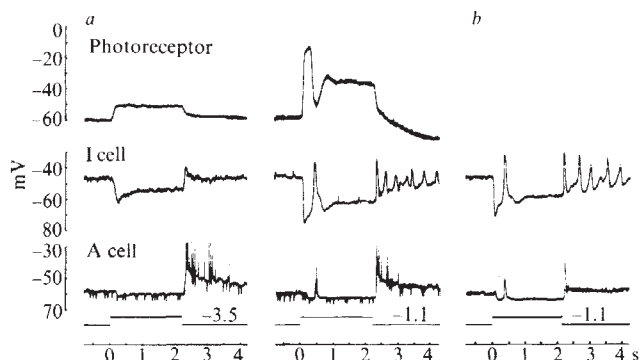
Many of the neurones of the supra-oesophageal ganglion respond to the dimming of light, and are probably involved in the complex defensive response of the animal to a shadow^{9,10}. Most cells do not respond to the onset of a pulse of light but only to its termination. Certain small cells near the photoreceptor terminals, however, respond to the onset as well as to the termination of light^{11,12}; at light onset these cells hyperpolarise, and at the termination of light they depolarise transiently. As these 'biphasically responding cells'¹³ sense both the onset and

the offset of light, they must precede other cells in the visual pathway. Indeed, their hyperpolarising and depolarising responses, as well as the photoreceptor responses, are spared when sodium action potentials are eliminated by tetrodotoxin (TTX), abolishing all activity in other ganglion cells.

We have investigated further the properties of biphasically responding cells and have found that they can be divided unambiguously into two types on the basis of their characteristic responses to light, their dark resting potentials, and their ability to generate sodium- or calcium-dependent action potentials. So far we have found only one cell of each type on each side of the ganglion.

The responses of a photoreceptor and of each type of ganglion cell to pulses of dim and bright light are presented in Fig. 1. The response of one type, the 'inverting cell' (I cell), is essentially a mirror-image of that of the receptor. Evidence that the I cell is a second-order neurone, in other words directly postsynaptic to the photoreceptors, will be presented below. The I cell displays a low resting potential in the dark (mean -45 mV; range -30 to -50 mV; $n=9$), a marked hyperpolarisation at the onset of light, and a depolarisation or oscillations at the termination of the light pulse. The second type of biphasically responding cell

Fig. 1 Responses to light of a photoreceptor, an I cell and an A cell. *a*, Responses in normal saline to dim (left-hand traces) and moderately bright (right-hand traces) pulses of light of 2 s duration. I cell record is atypical in that most I cells generate off-responses, even following bright lights, which consist of a single depolarisation overshooting the dark resting potential, rather than of the multiple oscillations shown here. *b*, Responses of the same cells shown in *a* when the ganglion was perfused with TTX 10^{-6} g ml⁻¹. The photoreceptor impulse was lost after changing solutions. The recordings from the photoreceptor and I cell were made simultaneously; the A cell responses are from another experiment. Light intensities (log mW cm⁻²) are indicated to the right of the shutter monitor trace (lower trace).



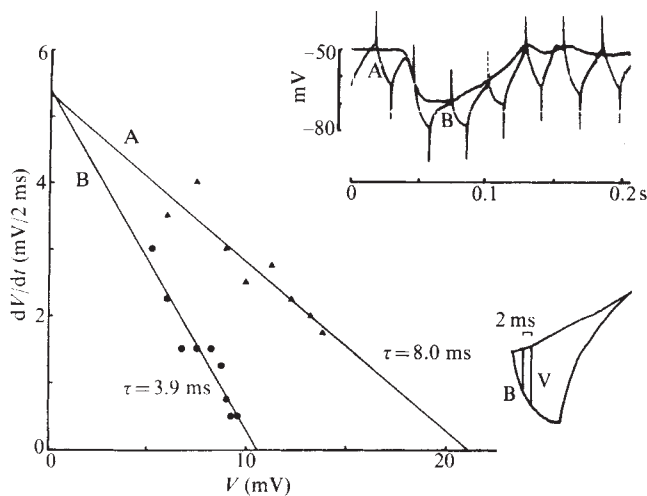


Fig. 2 Conductance of the I cell increases during its response to light. The experiment is illustrated at the top right. Two oscilloscope sweeps are superimposed. The voltage response to a flash of light was recorded by one sweep; short pulses of current of constant magnitude were superimposed on the flash response on the other sweep. The changes in voltage evoked by these current pulses were larger and had a longer time constant in the dark (for example, A) than during the light response (for example, B). To obtain a value for the time constant of the membrane during light and dark independent of bridge balance, a phase-plane plot was made relating the rate of change of the voltages (dV/dt) in pulses A and B to the voltage (V). Ordinate, dV/dt , measured as the difference between voltage measurements at 2 ms intervals during the rising phase of the hyperpolarising pulse (diagram, lower right). Abscissa, V , membrane potential of cell (see diagram). The exponential increase in the negative voltage in time is described by $V = V_{\infty}(1 - \exp(-t/\tau))$ where V_{∞} is the voltage developed at the end of a long current pulse and τ is the time constant. To relate V and dV/dt , one can take the time derivative of the above expression: $dV/dt = (V_{\infty}/\tau) \exp(-t/\tau)$. These two expressions can each be solved for $V_{\infty} \exp(-t/\tau)$ and equated. Therefore $dV/dt = -V/\tau + V_{\infty}/\tau$. This equation describes the lines drawn through the data points on the phase-plane plot by linear regression. From the slopes of each line the time constants of rise of pulses A and B were calculated using the above equation and are shown. As the time constant is inversely proportional to the conductance of the cell, the conductance is increased by a factor of about two.

has been called an 'amplifying cell' (A cell), as the most conspicuous feature of its response is a large depolarisation after termination of illumination even when the concurrent voltage changes in the receptors and I cells are small. The A cell has a resting potential (mean -64 mV; range -55 to -70 mV; $n = 17$) more typical of ganglion cells and the photoreceptor itself; it gives only a small hyperpolarisation to light, but a large depolarisation which sets up action potentials after termination of illumination. Previously published work has dealt with neurones with the properties of A cells¹¹⁻¹⁴ and has shown that impulses arising in these cells at the termination of a light pulse are conducted along the cells' axons in the contralateral connectives.

The hyperpolarising and depolarising responses to light of both I cells and A cells are preserved in TTX (Fig. 1b). In TTX, the A cell is no longer able to generate action potentials, and its background synaptic activity ceases.

One simple way to account for the observations presented in Fig. 1a and b is that the I and A cells are the second- and third-order neurones in the visual pathway. When depolarised by light, the photoreceptors release inhibitory transmitter on to the I cells, causing them to hyperpolarise. In turn, the I cells, when released from inhibition by dimming or by dark, depolarise and release excitatory transmitter on to the third cell in the sequence, the A cell. This scheme is consistent with the

observation that I cells in the dark rest at a relatively depolarised membrane potential which could cause them to release transmitter constantly. Such a circuit has been proposed by Ozawa *et al.*¹⁴ as one likely way of accounting for their observations on cells with the properties of A cells.

The proposed scheme implies that I cells would have a greater conductance in the light, when they are bombarded with inhibitory transmitter, than in the dark. A cells would display just the opposite properties: a greater conductance in the dark when they are bombarded with excitatory transmitter than in the light. We have found that the conductance of the I cell is indeed increased in response to light (Fig. 2). The conductance of cells with properties of A cells has been measured^{13,14} and was found to be greater in the dark than in the light. The direction of the conductance changes in these two biphasically responding cell types supports the hypothesis that the receptor neurone releases inhibitory transmitter in the light and the I cell releases excitatory transmitter in the dark.

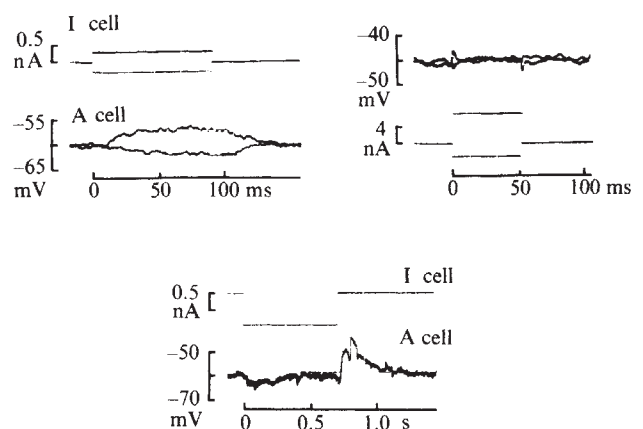
Recordings made simultaneously from an I cell and an A cell show that the I cell is presynaptic to the A cell. Injection of current in the dark into the I cell in either the depolarising or hyperpolarising direction causes a steady potential change of the same sign in the A cell (Fig. 3a). This result would be expected if polarisation of the I cell were increasing or decreasing a steady release of excitatory transmitter from this neurone. The long latency between I cell polarisation and A cell response suggests that transmission at this synapse is chemically mediated. Injection of current into the A cell causes no change in the I cell membrane potential (Fig. 3b). Thus, the I cell is presynaptic but not postsynaptic to the A cell. Hyperpolarising currents injected into the I cell to mimic the effect of light can cause a depolarisation in the A cell at the cessation of the current pulse (Fig. 3c), much like the off-response to light.

Functional membrane properties of I cells and A cells

Both I cells and A cells can display regenerative activity associated with repolarising potential changes in the photoreceptor (Fig. 1a and b). The nature of this regenerative activity is decidedly different, however, in the I cell and A cell.

The activity that one sees in the I cell ranges from oscillations to small, slow (about 20 ms to peak) regenerative events with noticeable inflections on the rising phases. This regenerative activity is insensitive to TTX (Fig. 1b). Hyperpolarising current pulses injected into the cell soma evoke oscillations at the break

Fig. 3 Depolarisation or hyperpolarisation of an I cell causes potential changes of the same sign in the contralateral A cell (upper sets of traces). Polarisation of the A cell does not affect the I cell (lower set of traces). The magnitude of the injected current was measured by recording the command potential which drove the current pump in the d.c. preamplifier. The experiment was carried out in the dark.



of the pulse (Fig. 4). Distinct action potentials of greater amplitude can be evoked if the preparation is bathed in 100 mM tetraethylammonium ion (TEA). This compound generally blocks voltage-sensitive potassium channels, thereby reducing outward potassium currents and allowing inward currents to generate larger voltage changes than in normal saline. The amplitudes and rates of rise of the action potentials generated in the presence of TEA are decreased when calcium is replaced by magnesium. When calcium is replaced by barium in the presence of TTX, an action potential can be generated even in the absence of TEA. Thus, the regenerative activity recorded from the soma of an I cell is due to voltage-sensitive calcium channels.

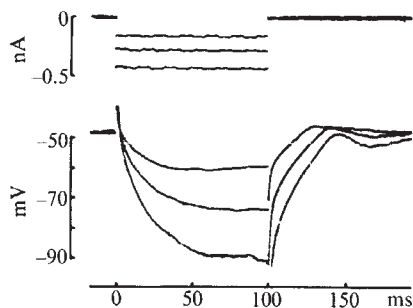


Fig. 4 Oscillations evoked in the I cell at the break of a hyperpolarising current pulse. Three pulses of increasing amplitude have been superimposed. Upper traces: current monitor, current recorded as in Fig. 3. Lower traces: membrane potential.

The impulses set up in the A cell are, on the other hand, abolished by TTX. A cell impulses are always distinct action potentials rather than oscillations, they are conducted along the cell's axon in the circum-oesophageal connective, and they have the fast rise time (1–2 ms) typical of sodium-dependent action potentials.

Information transfer at the first two synaptic stages

Both the peak and plateau of the I cell response (Figs 1 and 5a and b) grow over a range similar to that of the receptor response (ref. 5, Fig. 4). Thus, information concerning the intensity of maintained light is transferred from the receptor to the I cell. Figure 5a shows that the membrane potential of the I cell is a function of light intensity over a range of about 5 log units. The slope of this relationship varies widely among different I cells for reasons as yet unknown.

Figure 6 shows a simultaneous recording made from a photoreceptor and an I cell. The steep slope of the relationship between intensity and plateau response in this I cell shows that receptor potentials as small as 1 mV cause a response in the postsynaptic cell (Fig. 6a). For voltage changes in the receptor ranging from 1–20 mV, the membrane potential in the I cell was roughly proportional to the membrane potential of the photoreceptor (Fig. 6b).

In contrast to the plateau response of the I cell (Fig. 5a), that of the A cell saturates at about $10^{-3.6}$ mW cm $^{-2}$ (Fig. 5b). According to our hypothesis, the A cell plateau is the resting potential of the cell in the absence of synaptic input; at light intensity dimmer than $10^{-3.6}$ mW cm $^{-2}$, the A cell is depolarised by excitatory transmitter released from the I cell. In brighter lights the I cell seems to be hyperpolarised enough so that it stops releasing transmitter.

The essence of the A cell response to a light pulse is its depolarising off-transient. When the ganglion is bathed in TTX, this depolarisation no longer elicits action potentials and its amplitude can be measured. The amplitude of this off-transient is a function of both the intensity and the duration of the

preceding light pulse (Fig. 5d). The off-depolarisation increases with light intensity for dim lights and saturates at moderately bright light. As the amplitude of this saturated response, and the intensity at which it saturates, depends on the duration of the preceding light as well as on its intensity, information concerning absolute light intensity is not preserved at the A cell stage.

Discussion

The physiological observations presented above suggest that visual information passes serially from the photoreceptors of the median eye to decrementally conducting I cells and from them to A cells. The A cells transmit information about dimming to the ventral ganglion.

At the first integrative stage, the synapse between the photoreceptors and the I cells, the light response is inverted by an inhibitory synapse. I cells respond to changes of membrane potential in photoreceptors as small as 1 mV from dark resting potential (–60 mV). Thus, release of transmitter from the receptor must take place at membrane potentials more negative than those in squid presynaptic terminal¹⁵ and even takes place at the dark resting potential (B. Schnapp, personal communication). Release of transmitter at the well characterised synapses in the squid, lamprey and the hatchetfish nervous systems, is not detectable until the presynaptic cell has been depolarised by roughly 20 mV to about –45 mV (refs 15–17). The input–output relationship of the receptor synapse has therefore been shifted so that release, and by inference calcium entry¹⁸, occurs at more negative voltages.

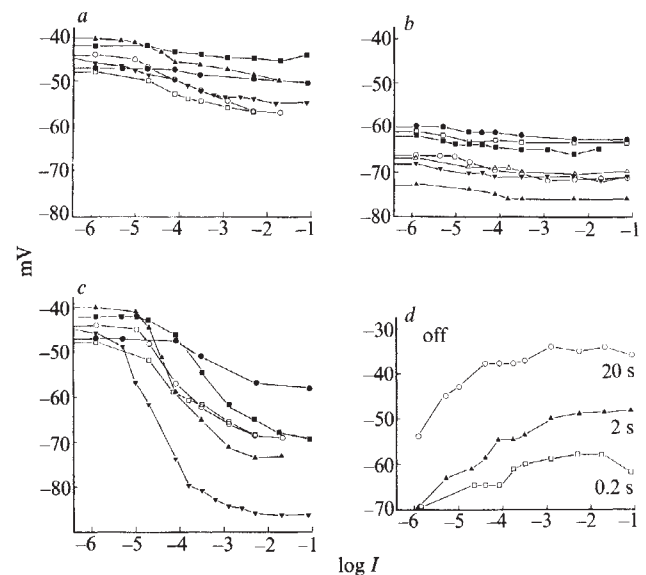


Fig. 5 Responses to light of I cells *a* and *c* and A cells (*b* and *d*). Plateau hyperpolarisations measured in six I cells (*a*) and seven A cells (*b*) just before the end of a 2-s light pulse and plotted as a function of light intensity. *c*, Peak hyperpolarisations of the I cells plotted as a function of light intensity. *d*, amplitudes of off-responses in an A cell (▼ in *b*) as a function of light intensity for light pulses of three different durations as indicated. The experiment illustrated in *d* was carried out in the presence of TTX.

Responses of the I cell to light also resemble those of bipolar cells of the vertebrate retina. In both, the responses are graded, the onset and termination of light evoke potential changes from dark rest in opposite directions to one another, and neither cell type normally generates all-or-none action potentials. Bipolar cells have been reported to exhibit oscillations in certain conditions^{19,20} which have the appearance of small action potentials but are graded in amplitude. These oscillations in the bipolar cell are similar to those sometimes seen in the I cell and might similarly be due to voltage-sensitive calcium channels.

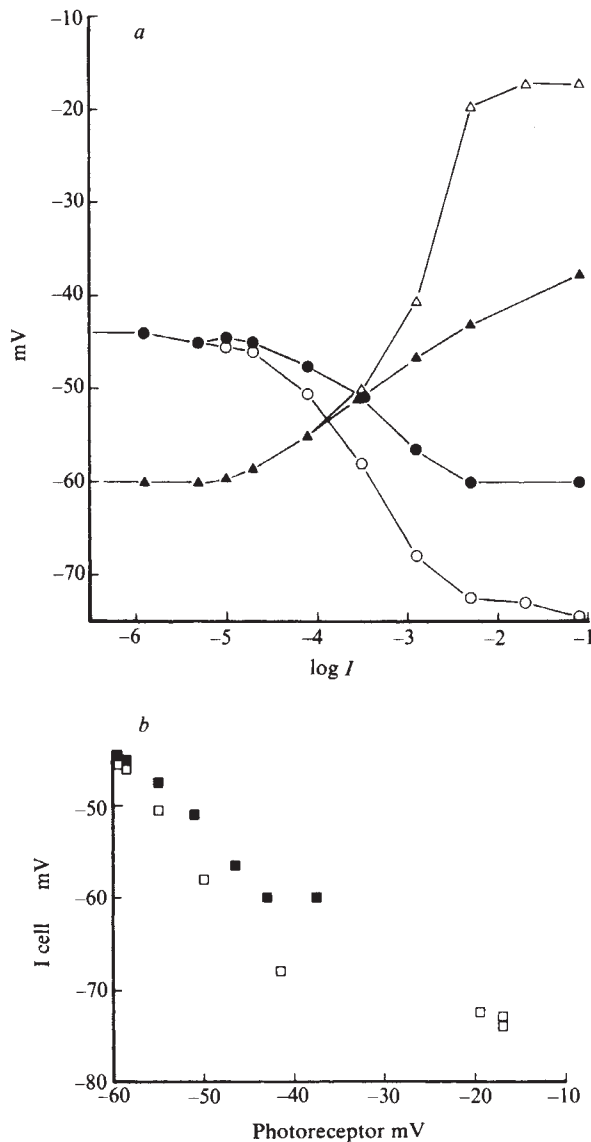


Fig. 6 Relationship between membrane potentials of an I cell and a photoreceptor recorded simultaneously. *a*, Peak (Δ) and plateau ($\blacktriangle$) depolarisations in the photoreceptor and peak ($\circ$) and plateau ($\bullet$) hyperpolarisations in the I cell as functions of light intensity. Plateaus were measured at the end of a 2-s light pulse. *b*, Plot of the membrane potential changes induced by light in the photoreceptor against changes in the I cell. The data from *a* are replotted showing relationship of peak ($\square$) and plateau ($\blacksquare$) measurements of light responses from the two cells. Dark resting potentials were -44 mV in the I cell and -60 mV in the photoreceptor. The steepness of the relationship is not significant, as it depends on the exact location of the electrode along the length of the photoreceptor axon (6.6 mm from the ganglion in this experiment). This I cell is represented as $\circ$ in Fig. 5a and c.

The responses of I cells also share features of second-order cells in insect visual systems. Intracellular recordings have been made from second-order neurones (laminar type I neurones²¹) in lateral eyes of several insects (locust²² and fly²³⁻²⁵) and in the median eye of the dragonfly²⁶. Light responses of second-order cells in these insects resemble light responses in the I cell in that they have a large, transient, initial hyperpolarisation which decays to a plateau.

It is not clear whether second-order neurones in insects generate action potentials. As action potentials have been seen occasionally in these insect cells²⁶, the absence of impulses from intracellular recordings is usually attributed to microelectrode damage^{23,26}. On one occasion, however, we have recorded

action potentials from I cells in normal saline which resembled those recorded from second-order cells of the dragonfly ocellus but which were insensitive to TTX. Perhaps the impulses recorded occasionally in insect second-order cells are calcium-dependent.

At the second integrative stage in the barnacle, the synapse from the I cell to the A cell, the intensity range over which the responses of A cells are graded is narrowed and the off-response is amplified (Fig. 5b). When I cells are released from a hyperpolarisation caused by light or by current, regenerative activity is seen. We have shown that this activity reflects the activation of voltage-sensitive calcium channels, and it is reasonable to assume that these channels are at least in part those associated with the release of transmitter. The onset of dark, then, causes depolarisation of the I cell, the activation of voltage-sensitive calcium channels, and the release of transmitter which excites the A cell. The release properties of this synapse are such that the graded portion of the light response is for the most part not transmitted to the third-order cell. The oscillation occurring in the I cell when the light goes off, on the other hand, is transmitted and amplified. The large depolarisation occurring in the A cell at the termination of light generates a burst of TTX-sensitive action potentials.

We have suggested that the I cell constantly releases transmitter on to the A cell in the dark. Like Ozawa *et al.*¹⁴ we have observed that in the dark the A cell membrane potential undergoes greater fluctuations than it does in the light. Ozawa *et al.*¹⁴ suggest that this increased noise arises from fluctuations in the continuous release of transmitter on to the cell in the dark and that in the light transmitter release stops.

We have observed excitatory and inhibitory synaptic potentials on both the I cell and the A cell in various conditions. The role of these inputs is not understood; they might well contribute to the light responses in I cells and A cells. It seems unlikely, however, that a third type of biphasically responding cell would be interposed between the receptor and I cell, or between the I cell and A cell, as in our many impalements of small cells in many preparations we have found only these two types of cells which respond when light is turned on. Final confirmation of our postulated scheme must come from the demonstration of direct contact between the cells with the electron microscope.

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Evidence for splicing of interrupted immunoglobulin variable and constant region sequences in nuclear RNA

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Evidence is presented that the mouse light-chain coding sequence is interrupted in a 27S nuclear RNA species, whereas the sequence is continuous in both a 13S nuclear RNA and in cytoplasmic mRNA. The discontinuity of coding regions in the 27S nuclear RNA parallels the situation found in myeloma DNA and indicates, therefore, that the removal of interruptions in the V and C regions occurs at the level of nuclear RNA.

IMMUNOGLOBULIN (Ig) polypeptides consist of variable (V) and constant (C) regions associated in a single molecule. This molecule is synthesised, by the antibody-producing cells, from a single mRNA molecule containing both V and C regions¹. Recent restriction enzyme mapping data^{2,3} on mouse κ class light (L) chains have shown that the germ-line DNA carries the V and C genes separated by an unknown stretch of DNA. Differentiation of antibody-producing cells seems to result in DNA rearrangement such that the V and C genes are moved closer together in the genome^{2,3}. It has been shown, however, that such a rearrangement does not result in a fully contiguous stretch of DNA carrying V and C_κ genes in a linear array³ but rather the active κ L-chain gene of myeloma cells contains an interruption sequence at or near the junction of the V and C regions. A similar interruption has also been observed in a mouse myeloma λ L-chain⁴. Therefore, the transcription unit encoding the V and C genes includes material which is not represented in the final mRNA.

Recently, data have been obtained from several other systems, showing the occurrence of discontinuous genes. These appear in adenovirus and SV40 (refs 5–11), ribosomal cistrons^{12–16}, globin genes^{17,18} and ovalbumin genes^{19,20}. One possible route by which this extra material may be removed is during the processing of the nuclear RNA (nRNA) precursors of mRNA. Thus, the entire Ig gene (including the sequence of the interruption) can be transcribed into a large nuclear RNA precursor followed by a process of ligation to form contiguous V and C regions. Alternatively, it is possible that the V and C regions could be transcribed into separate nRNA molecules from which the V and C genes are joined. A completely different type of process might involve the RNA polymerase 'jumping' across the interruption sequence (thereby effectively ignoring it) and generating contiguous sequences in RNA from discontinuous sequences in DNA. We have investigated the arrangement of V- and C- region sequences in the nuclear RNA of MOPC 21 cells and conclude that transcription of the interrupted Ig gene produces a nRNA molecule or molecules in which an interruption occurs at or near the V–C junction. Joining of this V–C interruption, therefore, apparently occurs at the level of nuclear RNA to generate a 13S nuclear RNA molecule with continuous V and C regions.

Characteristics of a 27S nRNA containing L-chain sequences

RNA was prepared from detergent-washed nuclei of mouse MOPC 21 cells, and fractionated on a sucrose gradient following

denaturation in dimethyl sulphoxide (DMSO). An aliquot of each gradient fraction was hybridised with ³²P-labelled L-chain complementary DNA (cDNA) and the single strand-specific S₁ nuclease-resistant radioactivity determined by trichloroacetic acid (TCA) precipitation (Fig. 1). The main peak of hybridisation observed was around 13S but significant hybridisation was found in regions up to about 30S (a minor but reproducible peak

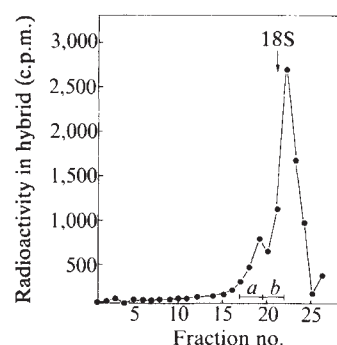


Fig. 1 Hybridisation of L-chain cDNA to fractionated mouse myeloma nuclear RNA. Nuclear RNA was prepared from mouse myeloma P3K cells (MOPC 21)²¹ by a modification of a previous procedure²². 10⁹ cells were lysed in 20 ml 0.5% NP40, 0.24 M sucrose, 10 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM KCl. The nuclei were pelleted and suspended in 1 ml 0.12 M sodium phosphate pH 6.8, and lysed with 100 ml 8 M urea, 0.24 M sodium phosphate pH 6.8, 1 mM EDTA, 0.5% SDS²³. 10 g hydroxyapatite (Biorad) were added and stirred vigorously for 10 min. The hydroxyapatite plus the DNA was removed by centrifugation (5 min at 1,000g) and the supernatant dialysed exhaustively against 0.25 M NH₄Ac, 1 mM EDTA, 0.5% SDS. RNA was recovered by ethanol precipitation and dissolved in 10 ml 7 M urea, 0.35 M NaCl, 0.5% SDS and 1 mM EDTA. This solution was extracted twice with an equal volume of buffer-equilibrated phenol-chloroform (1:1 v/v) and ethanol precipitated. The recovered RNA was washed twice with 3 M NaAc pH 6.0 (ref. 24) to remove residual DNA, reprecipitated from 100 mM NaAc pH 6.0, and finally dissolved in 1 mM EDTA, 70% DMSO. The solution was heated for 2 min at 70 °C, rapidly diluted with 3 vols 0.5% SDS, 100 mM NaCl, 1 mM EDTA and 10 mM Tris-HCl pH 7.5, and layered on 15–40% sucrose gradients (in 100 mM NaCl, 0.5% SDS, 10 mM Tris-HCl pH 7.5, 1 mM EDTA). Centrifugation was for 5 h at 38,000 r.p.m. in an MSE 6 × 14 ml rotor at 25 °C. The gradients were fractionated into 0.5-ml fractions and 100-μl aliquots were ethanol precipitated with 10 μg *Escherichia coli* DNA plus 2,500 c/m unfractionated ³²P-L-chain cDNA. The recovered precipitate was dissolved in 20 μl 0.3 M NaCl, 10 mM Tris-HCl pH 7.5, 0.5% SDS and 1 mM EDTA and incubated at 70 °C for 16 h. The hybridised cDNA was estimated by TCA precipitation following S₁ nuclease digestion as previously described³. The cDNA used in this study was made with MOPC 21 L-chain mRNA and T₂-G₃-T as primer. This primer initiates at the equivalent of residue 205 just within the C region^{25–27}, so cDNA molecules begin with C_κ sequences and proceed into the V region. The V+C cDNA used in Fig. 2 consisted of a cDNA band eluted from an acrylamide gel: this band was about 700 bases long and represents the maximum length transcript which can be obtained from the T₂-G₃-T priming site. Sedimentation is right to left. Pools a and b were selected for resedimentation.

of hybridisation being observed at around 27S). Evidently, therefore, the steady-state nRNA of MOPC 21 cells consists mainly of a species showing a similar sedimentation value to 13S cytoplasmic mRNA. A second species (around 27S) seems to be present in the nRNA but in rather low proportions: this RNA will be approximately two to three times the size of the L-chain mRNA and presumably represents a precursor of the L-chain mRNA. This conclusion is consistent with a recent publication²⁸ which showed that, although a 40S species is the probable initial transcript of the L-chain gene, a 13S species was the predominant L-chain-specific nuclear RNA present, with a 24S minor species also being observed.

We have investigated the spatial arrangement of the V and C regions in these nuclear RNA species with an assay previously used to demonstrate the presence of an interruption in the κ L-chain gene³. This assay consists of electrophoretic determination of the size of ³²P-cDNA, protected from S₁ nuclease digestion, after hybrid formation with DNA or RNA. The rationale of this approach is that cDNA containing V- and C-region sequences would be completely resistant to S₁ nuclease after hybridisation with RNA or DNA in which the V- and C-region sequences are continuous. On the other hand, this cDNA would be only partially resistant to S₁ nuclease when hybridised to RNA or DNA in which interruptions of V and C regions occur. In a previous study³ we found that V + C cDNA (that is, cDNA of about 560 bases in length, made as described in Fig. 1 legend, extending over both C and V regions) was not fully protected from S₁ nuclease when hybridised with myeloma DNA prepared from cells making the specific L-chain. Instead,

we observed a major band of protected cDNA (about 290 bases in length) corresponding approximately to the C region. These results indicated the presence of an interruption at or near the junction of V and C_κ regions and also indicated that the V_κ gene may possess interruption(s), as we failed to detect a cDNA fragment consistent with the expected V-gene length³. This assay provides a convenient method to study nuclear RNA precursors, as the S₁ nuclease protection of V + C cDNA by nuclear RNA will exhibit half protection of the type observed with myeloma DNA (DNA-like protection) if the nRNA precursor contains interruptions of the V and C regions or full protection if the nRNA precursor contains continuous V and C regions.

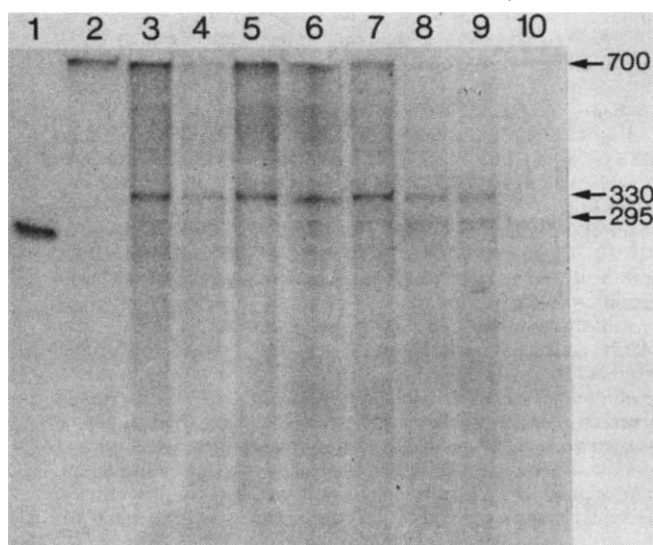
Figure 2 shows the pattern of S₁ nuclease protection of V + C cDNA (700 bases) obtained by hybridisation with various nRNA gradient fractions (from Fig. 1). The pattern of cDNA protected from S₁ nuclease by hybridisation with cytoplasmic mRNA shows two major bands (slot 3): one band of intact cDNA 700 bases in length (complete protection) and a further band of partial protection around 330 bases. This latter material does not occur when purified 13S L-chain mRNA is used³ and possibly results from a specific processing of the RNA. This protection pattern of the cDNA was also found in the higher molecular weight region of the fractionated nRNA but, in addition to this, a further component was observed, with a peak of sedimentation around 27S. This component consisted of an RNA species capable of only partially protecting the 700-base V + C cDNA (after hybridisation) to yield a protected cDNA fragment around 295 bases. This partially protected cDNA fragment is similar to the 'half protection' which we previously observed with experiments on myeloma DNA (that is, DNA-like protection)³ and presumably reflects interruption sequences present in the 27S RNA which are absent from the 13S mRNA. Therefore, in the nuclear RNA there are apparently two separate components, the first having a sedimentation value around 27S, possessing a DNA-like arrangement of V and C regions, and the second with a value around 13S and partly possessing continuous V and C regions (that is, mRNA-like arrangement of V and C regions). Thus, the primary transcripts of the κ -chain gene seem to possess the interruption which occurs at or near the V- and C-region junction.

Confirmation of interrupted V and C regions in the L-chain 27S nRNA

The sedimentation time used for the nRNA described in Figs 1 and 2 was short so as to display any putative nRNA molecules larger than 27S. The result was that the resolution of the 27S and 13S species was poor, and considerable overlap of the two patterns of S₁ nuclease protection was displayed by the various gradient fractions in Fig. 2. To increase the resolution of these molecules and to characterise the 27S molecule further, the two regions of the gradient shown in Fig. 1 (a and b) were pooled and resedimented after further denaturation in DMSO. The hybridisation profiles of L-chain cDNA with these various fractions are shown in Fig. 3. Both pools a and b, as expected, showed hybridisation with L-chain cDNA at a position consistent with a 13S RNA species, and in addition showed peaks of hybridisation at around 27S (pool a) and 20S (pool b). No hybridisation with L-chain cDNA was observed if the nRNA pools were boiled in alkali before resedimentation. Therefore, the material sedimenting at 27S is indeed a high molecular weight nRNA species containing L-chain sequences. The only 'steady-state' precursor molecule which we observe is the 27S molecule, whereas the nature of the 13S nRNA is more uncertain. This RNA is probably a true nuclear species but could be partly contamination of nuclear RNA by 13S cytoplasmic mRNA.

The pattern of S₁ nuclease protection of cDNA after hybridisation to the resedimented nRNA pools (a₁, b₁, and a₂ + b₂, Fig. 3) clearly shows the difference between the 27S and the 13S nRNA, as cross-contamination of the 27S RNA with 13S RNA

Fig. 2 Pattern of S₁ nuclease protection of L-chain cDNA after hybridisation with fractionated mouse myeloma nRNA. 100-μl aliquots of fractions 17–23 (from Fig. 1) plus 100-μl aliquots of cytoplasmic mRNA were hybridised to V + C cDNA as in Fig. 1. Following hybridisation, the nucleic acids were precipitated with ethanol and recovered by centrifugation. The pellets were washed with 70% aqueous alcohol, dried *in vacuo* and dissolved in 20 μl 0.28 M NaCl, 30 mM NaAc pH 4.5, and 4.5 mM ZnSO₄ (ref. 29). S₁ nuclease digestion was carried out for 1 h at 37 °C. Following digestion, the mixtures were diluted fivefold with 0.2 M sodium acetate pH 5.0, 5 mM EDTA and 20 μg ml⁻¹ *E. coli* tRNA, and ethanol precipitated. The recovered pellet was washed once with 70% alcohol, dried and dissolved in 20 μl 95% formamide by boiling for 3 min. The denatured samples were electrophoresed in 4% polyacrylamide gel in 8 M urea and the bands visualised by exposure to prefogged Fuji X-ray film at -70 °C using a fast tungstate intensifying screen. Slot 1 is a marker cDNA fraction of ~280 bases; slot 2 is the unhybridised 700-base V + C cDNA; slot 3 is the S₁ protection pattern of cDNA after hybridisation to cytoplasmic mRNA; slots 4–10 show the S₁ protection pattern of cDNA after hybridisation to gradient fractions 23–17 (that is, fraction 23 is the low molecular weight region of the gradient and fraction 17 is the high molecular weight region).



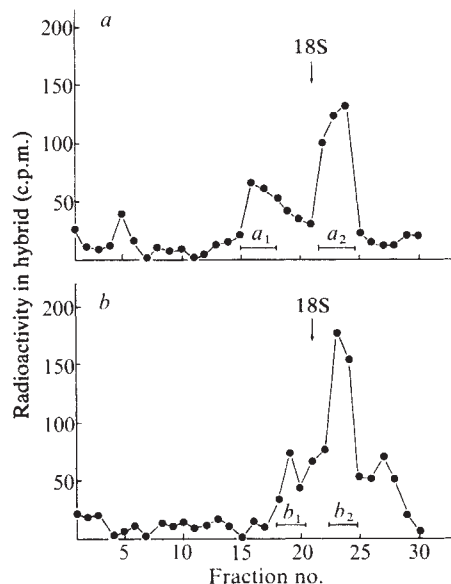


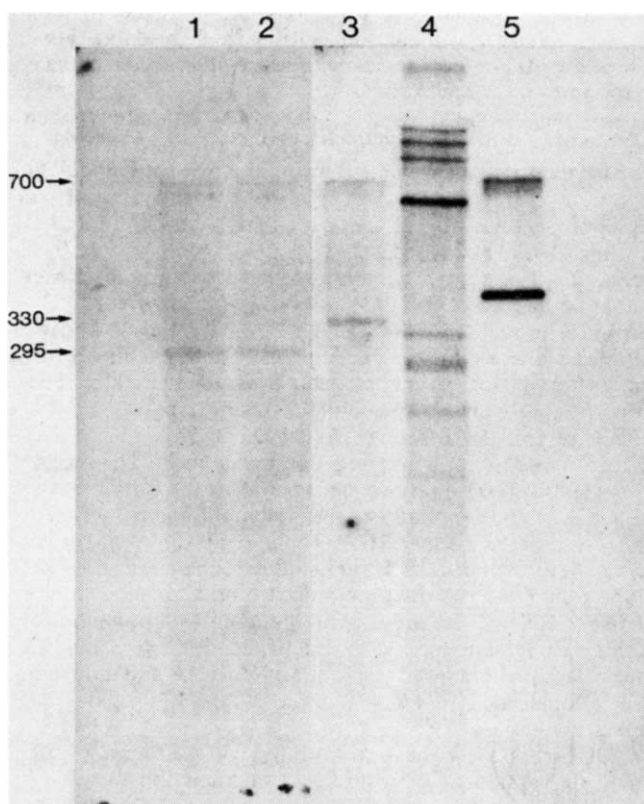
Fig. 3 Hybridisation profiles of cDNA with resedimented nRNA pools. The regions of *a* and *b* shown in Fig. 1 were pooled and the RNA recovered by ethanol precipitation. The RNA was denatured in DMSO and resedimented as in Fig. 1. Aliquots were taken and hybridised with unfractionated L-chain cDNA as in Fig. 1 followed by estimation of S_1 nuclease-resistant cDNA (TCA-precipitable counts). Sedimentation is right to left. The upper panel shows the hybridisation pattern of pool *a* and the lower panel that of pool *b*. The regions a_1 , b_1 and $a_2 + b_2$ were selected for further study.

was limited after the resedimentation. In addition, this experiment should be viewed in relation to the ability of purified 13S mRNA to protect *V + C* cDNA fully from S_1 nuclease³. Figure 4 shows the size of cDNA protected from S_1 nuclease after hybridisation of *V + C* cDNA (700 bases) to the nRNA pools. Both the 27S (a_1) and 20S (b_1) pools exhibit DNA-like protection patterns in that the major protected component of the *V + C* cDNA (initially 700 bases) was about 295 bases long. (This size estimate is based on a comparison with radioactive *Hae*III digestion fragments of Φ X174 which were run in a parallel slot of the gel.) The principal feature of these protection patterns is, therefore, the ability of the 27S nRNA to yield a protected cDNA fragment of about 295 bases in length which parallels the situation with myeloma DNA. The result, therefore, demonstrates that a sequence occurs in the initial transcripts, of the κ gene, which is absent from the mRNA. A small amount of intact *V + C* cDNA is observed after hybridisation with the 27S nRNA: this material may result from contamination with mRNA-like sequences and/or incomplete digestion of the cDNA. The 13S pool ($a_2 + b_2$) shows a different pattern of protection, yielding two bands of approximately equal quantities (700 and 330 nucleotides) of which the fully protected *V + C* 700-base component reflects the fully integrated state of at least part of the 13S nRNA. There is no evidence that the 295-base component results from hybridisation of cDNA to 13S nRNA, indicating that splicing events have occurred to eliminate this interruption at around the *V-C* junction. The origin of the 330 component is at present obscure, but as we were unable to detect such a component in purified L-chain mRNA³, we feel that the component is derived from nuclear RNA. We are, therefore, investigating the possibility that this material is a consequence of the nuclear RNA splicing which may involve, for example, putative interruptions in the V_κ -gene sequence.

To provide independent confirmatory evidence for the presence of interruption sequences in the 27S nRNA, we have compared the nucleotide sequences of the 13S mRNA and 27S nRNA in the area of the *V-C* junction (Fig. 5). To facilitate this comparison, cDNA was made complementary only to the C_κ region by using T_2 - G_3 -T primer in a reverse transcription using limiting concentrations of dCTP (0.05 μ M). The cDNA which

results from this reaction is mainly 100–200 bases in length. As the T_2 - G_3 -T initiates just within the *C* region^{25–27}, material less than 298 residues in length is complementary only to C_κ sequences. Thus, when the C_κ cDNA is hybridised to the C_κ sequences of either mRNA or nRNA and re-extended using the hybrid RNA as a new template, the sequence of the extended cDNA will be governed by the sequence of the new template. By incorporating the dideoxynucleotides as chain terminators in the re-extension transcription³⁰, we can generate a series of sequence-specific stopping points which can be visualised by fractionation of the resultant cDNA on acrylamide gels³¹. Figure 5 shows this sequence comparison using mRNA or 27S nRNA as template and dideoxy-TTP as the chain terminator. Clearly, the cDNA has increased in size as a result of annealing and re-extension on the RNAs (slots 2 and 3) compared to the starting cDNA population (slot 1). By comparison with the sequence of the mouse C_κ region²⁷, we can approximately position the bands on the gel with respect to amino acid residues in the protein. For reference in Fig. 5 an arrow has been marked at the T residue present in the codon corresponding to amino acid 110 (Asp) which is close to the *V-C* junction as defined by amino acid sequence data. Therefore, the sequence approximately up to this residue is C_κ -region sequence. Clearly, the cDNA band patterns resulting from extension on the mRNA and on the 27S nuclear RNA are identical up to around this residue, demonstrating that the C_κ sequences of these RNA species are identical. In addition, this result shows the lack of interruptions in the C_κ sequence of the nuclear RNA (at least

Fig. 4 The pattern of S_1 nuclease protection of L-chain cDNA by resedimented nRNA pools. Pools a_1 , b_1 and $a_2 + b_2$ (from Fig. 3) were ethanol precipitated, hybridised with *V + C* cDNA, digested with S_1 nuclease and fractionated on a polyacrylamide gel to visualise the protected cDNA fractions as in Fig. 2. The sizes of the various cDNA bands were calibrated using denatured nick-translated *Hae*III-digested Φ X174 (slot 4) and a 362-base fragment of *Xenopus* 5S DNA (slot 5). Mixed with the latter fragment in slot 5 was a sample of the *V + C* cDNA (700 bases). The other slots show the S_1 protection pattern of pool a_1 (slot 1), pool b_1 (slot 2) and pools $a_2 + b_2$ (slot 3). Slots 1 plus 2 and 3 to 5 have been exposed for different times for maximum display.



between the equivalent of amino acids 110 and 205). However, the cDNA band patterns obtained using the 27S nRNA clearly begins to differ from that obtained with the cytoplasmic mRNA at a position which approximates to the V-C junction. This difference of band pattern therefore reflects a difference of base sequence of the two RNA species after this area of the sequence. Thus, the result confirms unequivocally the presence of a sequence in the 27S nRNA which is absent from the mRNA. This interruption sequence is presumably adjacent to the C_κ region, which means that the MOPC 21 V region is not adjacent to the C_κ in this 27S nRNA. Localisation of the precise point of sequence divergence in the two RNA species must await actual sequence data on the 27S nRNA itself.

Two steps in the integration of V and C genes

The results described indicate that two RNA populations can be isolated from the same nuclei of MOPC 21 cells; one of these nRNA species exhibits a sequence arrangement of V and C genes similar to that in mRNA (that is, continuous V and C regions), and the other nRNA (27S) exhibits the interrupted arrangement of V and C regions which occurs in the DNA itself. The most likely scheme of events would, therefore, seem to be that the 27S species is a precursor of the 13S nRNA and that the final removal of interruptions from V and C regions occurs in processing events of nRNA. Evidence in favour of the 27S molecule as a precursor of L-chain mRNA comes from pulse-labelling experiments²⁸. In these experiments a shortlived 40S nRNA was found to be the initial transcript of the *Ig* gene in MOPC 21 cells. This 40S molecule was shown to be the precursor of a 24S molecule, and in turn the 24S RNA was found to be the precursor of the 13S nRNA. This 24S nRNA species is presumably equivalent to the 27S nRNA molecule discussed here and thus, this 27S molecule would be the precursor of the 13S nRNA and cytoplasmic mRNA.

It is impossible to say whether V and C regions are on the same molecule or whether the 27S fraction contains one molecule with the C region and another molecule with the V region. One piece of indirect evidence suggests that the V and C regions are on the same molecule. This evidence comes from experiments with myeloma/spleen cell hybrids³² in which, for example, there is no mixing of the C_H sequences and the V_H sequences, from the two parental cells, in the hybrid line. Final resolution of this point however, must await further characterisation of the 27S nRNA.

Integration of the V and C genes has commonly been supposed to occur at the level of DNA. We now know that this is only partially true and are able to present a two-step model for integration of V and C_κ genes (Fig. 6). The first step of the integration seems to be at the level of DNA. The inherited germ-line DNA carries separate V and C_κ genes which, by a process of DNA rearrangement, seem to be brought closer together in the DNA of the antibody-producing cell^{2,3}. An interruption remains in the DNA at or near the V and C_κ junction even after this rearrangement³. These events are the classical 'integration' events as defined by genetics. This relocation of the *Ig* genes would be the mechanism by which a cell becomes committed to produce a particular V gene and may also be responsible for activating the *Ig* gene itself. The second step of the integration process occurs after transcription of the gene and involves elimination of the interruption sequences of V and C genes in the nuclear RNA. The initial transcript(s) of the active *Ig* gene includes the V region, the interruption sequence and the C region. The discontinuous V and C_κ regions of the precursor nRNA(s) are presumably joined by a looping out of the intervening sequence followed by ligation of V and C_κ regions. This process gives rise to the 13S nRNA and in turn to active cytoplasmic mRNA where the V and C_κ regions are continuous.

In this discussion, we have specifically referred to the splicing of the interruption at or near the V-C junction by nRNA processing. This processing scheme will also probably be

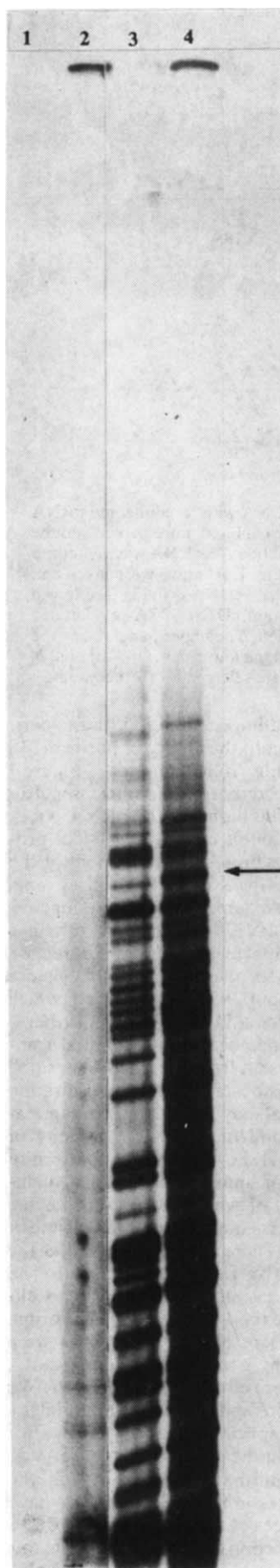


Fig. 5 Comparison of the sequences near the V-C junction of 13S mRNA and 27S nRNA. ³²P-cDNA was prepared with reverse transcriptase using MOPC 21 κ mRNA as template and T₂-G₃-T as primer^{26,27}, with 0.05 μ M dCTP and 10 μ M dGTP, dTTP plus 4 μ M ³²P-dATP. After incubation for 30 min at 37 °C the reaction mix was boiled for 5 min with 0.5 M NaOH neutralised with 1 M NaH₂PO₄, and the cDNA purified by chromatography on Sephadex G-100. The cDNA was annealed with either κ mRNA or 27S nRNA in 100 mM NaCl at 60 °C for 1 h. The cDNA was re-extended in the RNA:cDNA hybrid with reverse transcriptase in fresh reactions containing 100 μ M dATP, dCTP, dTTP and dGTP plus 10 μ M dideoxy-TTP³⁰ for 30 min at 37 °C and fractionated on 8% polyacrylamide gel in 8 M urea³¹. Slot 1, ³²P- Φ X174 digested with *Hae*III; slot 2, ³²P-cDNA starting material (not re-extended); slot 3, ³²P-cDNA re-extended on 13S messenger RNA; slot 4, ³²P-cDNA re-extended on 27S nuclear RNA. The arrows represent the position of a T residue in the codon equivalent to amino acid residue 110.

responsible for removing other interruptions which may occur in *Ig* genes. It has recently been shown, for example, that a germ-line V_{λ} gene possesses a short interruption in its linear sequence³³, and it is possible that V_{κ} genes possess insertions, as we were unable to detect a complete *V*-region size fragment in S_1 nuclease protection experiments with either MOPC 21 DNA³ or the 27S nRNA described here. It is also likely that nuclear RNA processing will be a general process for removal of the interruptions which have been found in other eukaryotic genes and in viral systems. Indeed, it has recently been shown that a globin nuclear RNA precursor possesses an interruption sequence which is absent from the mRNA³⁴.

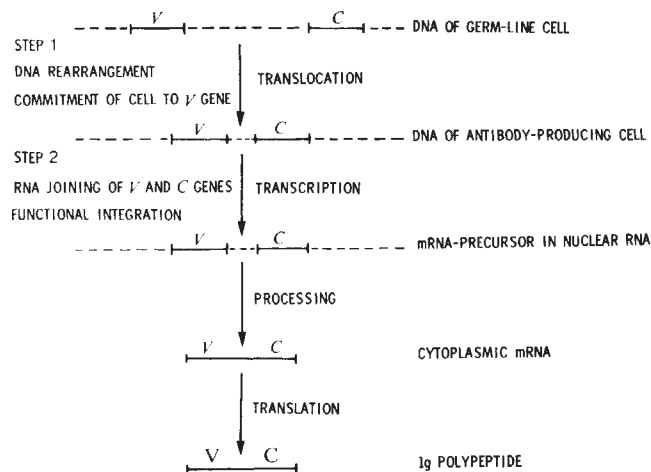


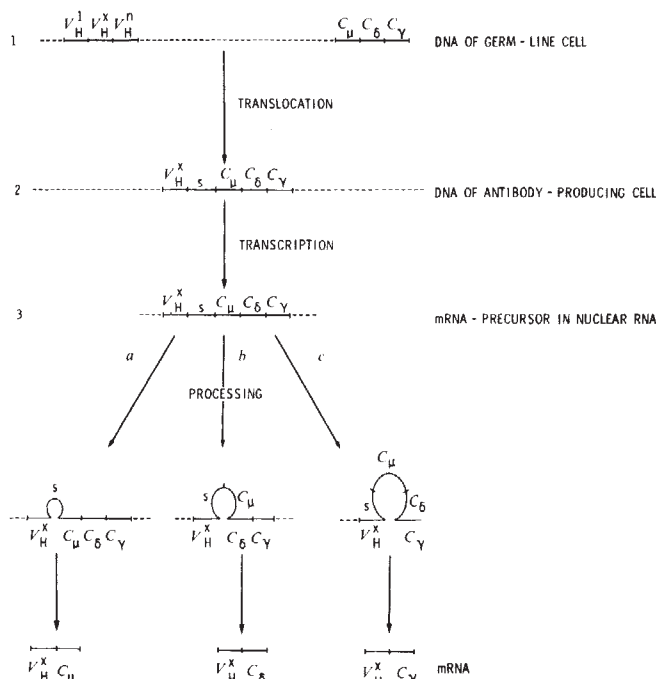
Fig. 6 Two-step model for integration of variable and constant region genes. Step 1, the inherited germ-line DNA carries separate *V* and *C* genes. These genes are brought closer together in the antibody-producing cell, but an interruption at or near the *V*-*C* junction remains in this DNA. This rearrangement or translocation of DNA is the classical 'integration' event and it is the event which allows the individual antibody-producing cell to become committed to a *V* gene. Step 2, transcription of the activated gene occurs to produce a nuclear RNA precursor which encompasses the *V* and *C* regions plus the interruption sequence(s). Functional integration of the *V* and *C* regions occurs during the processing of the precursor molecule, presumably by a looping out of the interruption sequence(s) followed by ligation of *V* and *C* regions. The sequence arrangement of the mRNA is thus achieved and from this the Ig polypeptide is made. It is predicted that any further insertions (such as may exist in the *V* region) will also be eliminated by nuclear RNA splicing.

Hypothetical scheme for Ig heavy-chain gene expression

The joining of *V* and *C* genes in the nuclear RNA may well explain some of the problematical features of Ig heavy(H)-chain gene expression. During the immune response there is a shift from IgM to IgG production, which seems to be the result of a switch of H-chain class within single clones^{35,36}. It has been shown that a single myeloma patient produces an identical V_H sequence in IgM and IgG^{37,38}, and other studies have shown that single cells apparently produce identical V_H regions in association with C_{μ} and C_{δ} (refs 39, 40). Thus, a cell becomes committed to a heavy-chain *V* gene and this same *V* gene can, at various times, associate with C_{μ} , C_{γ} and C_{δ} . It is also possible that a further association with C_{α} can occur, as an identical V_H sequence has been found in a myeloma patient producing IgG and IgA⁴¹. There does not seem to be a recommitment of the antibody specificity of a given cell, and previous models have envisaged either successional insertion of a V_H gene to the different C_H genes³⁷ or simultaneous insertion of copies of the V_H gene with the C_H genes⁴². A further model may now be proposed in which the processing of heterogeneous nuclear RNA is the site of association of the 'committed' V_H gene with the various C_H genes (Fig. 7). Thus, a single V_H gene is selected

from the V_H -gene pool and DNA rearrangement occurs to bring the V_H gene to the proximity of the C_H -gene array. Primary transcription of the active H-chain gene unit may now occur to produce a giant nuclear RNA precursor containing the V_H region and the various C_H -gene classes. The cell can now produce from one nuclear RNA precursor species the same V_H region associated with any of the C_H classes by processes of looping out intervening sequences, ligation of the V_H and C_H regions, followed by degradation of the unwanted RNA. A single cell, therefore, using such an nRNA precursor, could produce simultaneously or sequentially one, two or three mRNA species containing the same V_H sequence with the different C_H sequences. As shown in Fig. 7, this is a minimal model involving only the C_{μ} , C_{δ} and C_{γ} genes: there are some data suggesting the involvement of C_{α} but little evidence for involvement of C_{ϵ} . The minimal model can, however, easily be adapted to include these C_H genes if necessary by simply hypothesising their inclusion in the giant nRNA molecule. A general

Fig. 7 Hypothetical scheme for expression of a single heavy-chain V_H gene with multiple C_H genes. (1) The V_H -gene pool is depicted as a set of genes linked to the C_H -gene array. The order of the C_H genes is hypothetical and for simplification no subclasses of the C_H genes are shown. A single V gene (V_H^X) is selected for expression and DNA rearrangement occurs to bring the V_H^X and C_H genes closer together. A *V*-*C* interruption sequence may or may not exist between V_H^X and C_H (depicted as *S*). This process of rearrangement will commit the cell to a V_H gene and may also activate the H-chain transcription unit. (2) Transcription of the H-chain unit produces a giant nRNA precursor encompassing the V_H^X -gene *V*-*C* interruption sequence (if this occurs) and the C_H genes. (3) Intracellular processing of this giant nRNA molecule can give rise to any combination of V_H^X and C_H in mRNA. Thus, (3a) V_H^X mRNA can be generated by looping out of the *V*-*C* interruption sequence, ligation of V_H^X and C_{μ} followed by degradation of the remaining RNA; (3b) $V_H^X C_{\delta}$ mRNA by looping out sequence '*S*' plus the C_{μ} sequence followed by ligation of V with C_{δ} ; (3c) $V_H^X C_{\gamma}$ mRNA can be made from the same nRNA precursor by analogous processing events. A single cell can therefore simultaneously make one, two or three mRNA species with the same V_H gene and different C_H genes. 'Spacer' sequences must occur between the C_H genes to allow specificity of processing: these putative sequences would, of course, be eliminated by the nRNA processing. The model shown is a minimal model, as it excludes C_{α} and C_{ϵ} . It is possible that C_{α} (and C_{ϵ}) could also be involved in the pathway, in which case they can readily be accommodated by adapting the hypothetical giant nRNA precursor to include one or both of these C_H genes.



feature of the model is that it makes very clear predictions which can be tested experimentally.

In the way described, a single event (that is, the event responsible for V_H -gene commitment) is required for both activation of the heavy-chain gene transcriptions and multiple expression of the same V_H gene with any of the C_H genes at the level of mRNA. How is the required specificity of RNA joining likely to be achieved with a 'multicistronic' nRNA precursor of this type? Presumably, this joining is mediated through an enzyme or enzymes which can be either specific for the different genes or nonspecific, relying on RNA folding properties to achieve specificity. If specific enzymes are used it means that a large array of different species must exist, whereas nonspecific enzymes require only intrinsic properties of the gene sequence itself for correct joining. There are as yet no clues about the processes of RNA joining but doubtless, *in vitro* systems will be useful in exploring this aspect of RNA joining.

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letters to nature

X-ray emission from the companion to V861Sco

THE Copernicus satellite has observed V861Sco (Hr6283, Spectrum; $B0.5$ Ia, $V = 6.2$, $B - V = +0.28$, $U - V = -0.72$) as part of a programme devoted to the study of mass transfer in binary star systems. The programme described here uses the Princeton UV telescope¹ to make detailed measurements of absorption lines in the UV spectra of such stars. X-ray measurements are made simultaneously with a co-aligned proportional counter².

Significant signals from the X-ray detector were obtained during the first observation from 24 to 26 April 1978. The flux recorded, averaged over 1.2-h intervals, is shown in Fig. 1. At the end of the observation on 26 April a significant fall in intensity was observed. The hypothesis that this fall in intensity was caused by an eclipse of the X-ray source, was tested by a second observation which began on 27 May 1978. Results of this second observation are shown in Fig. 2. The flux was observed to decline at a time consistent with the known 7.8-d period⁷ of V861Sco. The low flux state lasts at least 1.3 d and the decline to this level occurred over a 0.7-d period.

There is no evidence of regular modulation (as is common with neutron star sources) greater than 15% peak to mean flux in the range 3–40 min.

The X-ray spectrum, observed when the signal was at maximum intensity, is shown in Fig. 3. Appreciable photoelectric absorption is present. The column density is equivalent to

$(4 \pm 1) \times 10^{23}$ H atoms assuming normal cosmic abundances and a thermal spectrum with a temperature equivalent to 11 ± 4 keV. The interstellar column of both atomic and molecular hydrogen to this region is $(2.3 \pm 1.0) \times 10^{21}$ atoms³. There is, therefore, considerable photoelectric absorption intrinsic to the source.

The Princeton guidance system maintains the pointing accuracy of both instruments to better than 0.1 arc s throughout the observing period. However, the beam width of the X-ray detector is 2.5° by 3.5° , full width at half maximum (FWHM), so the field has been examined for catalogued sources. No known sources were within the field with the exception of 4U1702–42 which was 2.8° from the centre for the V861Sco observations. 4U1702–42 is catalogued⁴ at 30 Uhuru flux units and would only contribute 3% or less than 1 OAO count in 62.9 s (the Crab nebula gives 375 OAO counts in this integration period). The signals observed by the Copernicus instrument thus indicate the presence of a new source which has been designated OAO 1653–40 (ref. 5).

The variable star V861Sco was shown by Walker to be a spectroscopic binary with a 7.8-d period and a mass function of 0.487 solar masses ($M_\odot$). A collapsed object was suggested as a possibility for the unseen companion⁷. Although Walker assumed the primary to have a mass 10 times that of the Sun ($10 M_\odot$), more reasonable estimates would be 20–30 $M_\odot$, implying a mass of 7–11 $M_\odot$ for the secondary, the actual value depending on the inclination of the system. This range is above the limit for a neutron star indicating that the compact secondary is a black hole.

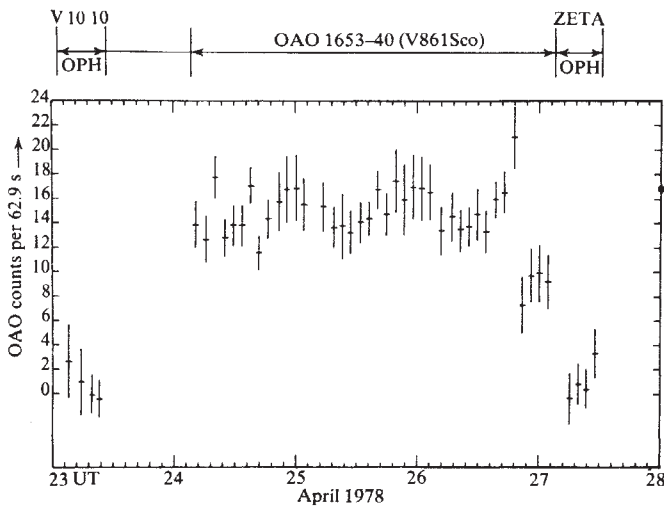


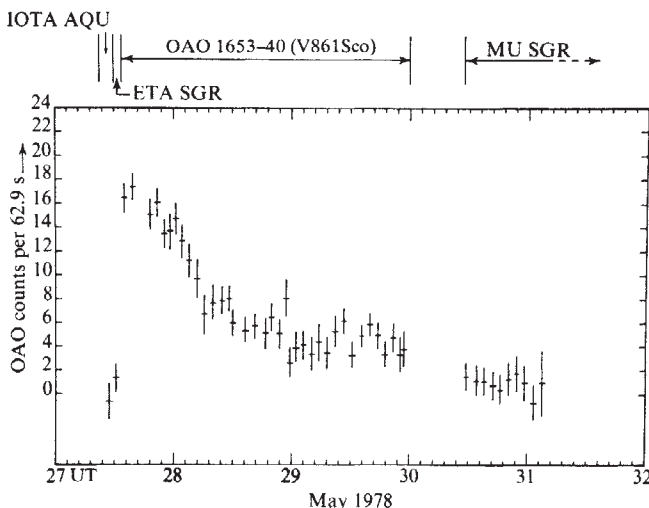
Fig. 1 First observation of V861Sco. The significant drop in the X-ray signal can be seen at the end of the observation. Times of observation of other target stars (used for background determination) are also indicated.

The X-ray observations support the identification of OAO 1653-40 with V861Sco. The two reductions in signal are separated by exactly four cycles of the binary star orbit and this is the strongest evidence for the suggested identification. A new orbital ephemeris is required to relate the phasing of the X-ray and optical data. UV observations obtained in three spectral regions ($\lambda\lambda$ 1110-1130, 1167-1185 and 1230-1247) during the X-ray eclipse indicate a stellar spectrum similar to that of κ Ori. Broad, strong, violet-displaced (by about -700 to -800 km s $^{-1}$) absorption components are seen in C III λ 1176, N V λ 1238 and λ 1242. Weak NV emission may also be present. These observations are, however, insufficient to relate the phases of the X-ray source and V861Sco.

The observed X-ray luminosity (3.8-8.8 keV and assuming a Crab-like spectrum) is given by $4 \times 10^{34} R^2$ erg s $^{-1}$ where R is the distance to the source in kpc. V861Sco is a member of the association Sco OB1 (ref. 8) and using the distance (1.95 kpc) to this cluster we obtain an X-ray luminosity of 1.5×10^{35} erg s $^{-1}$. The emission outside this energy band and electron scattering near the source increase the estimate of luminosity, possibly by several orders of magnitude.

When the X-ray source is in eclipse there is a significant residual signal at approximately 0.25 the non-eclipsed value.

Fig. 2 Second observation of V861Sco in which the expected eclipse is observed after four cycles of the binary star had passed since the first observation.



Further observations are needed to clarify the nature of this; for example, it may be a contaminating point source or arise from a possible supernova remnant in this association⁹.

The observed properties of OAO 1653-40 are so similar to those described for 4U1702-42 (ref. 4), that one might question whether the position of the latter is in error. Preliminary analysis of more recent data, obtained in July 1978, indicates that the intensity of the X-ray source has reduced considerably, implying that the source is transient within the sensitivity limits

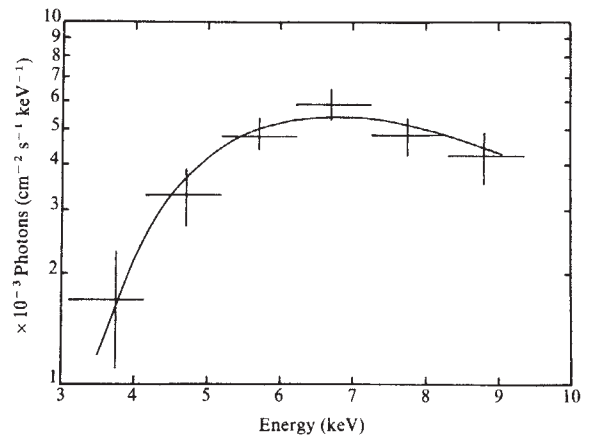


Fig. 3 The observed X-ray spectrum when the signal is at maximum intensity. A thermal spectrum has been fitted to the data but other spectral forms are not precluded. Significant photoelectric absorption is evident. Solid curve, thermal spectrum:

$$P(E) = \frac{(0.120 \pm 0.005)}{E} \exp\left(\frac{-E}{11 \pm 4}\right) \exp\left[(4 \pm 1) \times 10^{23} \sigma_{BG}\right]$$

(σ_{BG} are the Brown and Gould¹⁰ cross-sections for interstellar absorption).

of the Copernicus detector. If the association of the X-ray source with V861 is correct then either the known variable mass flow⁶ has caused further obscuration or has reduced accretion which has diminished the X-ray output.

We thank N. E. White for providing the constraints to regular pulsations.

Note added in proof: Clarification of the optical ephemeris and additional X-ray measurements suggest the observed decreases in flux are caused by the 'eclipse' of the X-ray source by a dense gas stream in the system. A true eclipse of the X-ray source by the primary star has recently been observed with Copernicus in further observations.

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COS B observation of high-energy γ radiation from 3C273

THE discovery of a high-energy γ -ray source at $\alpha(1950) = 12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$, $\delta(1950) = +3^{\circ} \pm 1.5^{\circ}$ is reported here. Arguments are given for the identification with 3C273. If this identification is correct, the γ -ray luminosity of 3C273 in the energy range 50–500 MeV is $2 \times 10^{46} \text{ erg s}^{-1}$ for $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

The majority of the reported γ -ray sources are galactic, as can be clearly established from their latitude distribution¹. The lack of unambiguous identification (with the exception of CG185–5 with PSR0531+21 and of CG263–2 with PSR0833–45) leaves a wide range of speculations as to the classification of the sources and to the nature of the emission mechanisms. The outstanding signature of these sources is the fact that their γ -ray luminosity ($>50 \text{ MeV}$) exceeds at least the radio and X-ray luminosities. Besides the continuing search for galactic γ -ray sources, some observations of the ESA γ -ray observatory COS B have been devoted to extragalactic targets². The region of the sky centred on $\alpha = 12^{\text{h}} 24^{\text{m}}$, $\delta = +7^{\circ}$ with an effective field of view of about 20° radius was observed from 24 May to 24 June 1976. This observation was particularly aimed at a sensitive search for high-energy γ -ray emission from M87 and 3C273.

The data have been analysed using a cross-correlation method: the frequency distribution of arrival directions of γ rays was correlated with the distribution expected for a point source as determined by calibration and confirmed by flight data on the strong source PSR0833–45 (ref. 2). The resulting correlation map follows the distribution expected for random data, apart from one clear maximum, which has a probability of 10^{-5} of being caused by random fluctuations at any position in the map. This excess follows the distribution expected for a point source located within a 90% probability error box defined by $\alpha(1950) = 12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$, $\delta(1950) = +3^{\circ} \pm 1.5^{\circ}$. Spectral information was obtained by analysing the data in two energy ranges. In each case a significant excess within the same error box was found. The derived characteristics of this source (designated CG291+65) are summarised in Table 1. The errors quoted include the estimated systematic errors.

Table 1 Characteristics of CG291+65

Position	$\alpha(1950) = 12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$, $\delta(1950) = +3^{\circ} \pm 1.5^{\circ}$		
Intensity	50–150 MeV	$(1.2 \pm 0.4) \times 10^{-6}$	photons $\text{cm}^{-2} \text{ s}^{-1}$
	150–500 MeV	$(0.3 \pm 0.1) \times 10^{-6}$	photons $\text{cm}^{-2} \text{ s}^{-1}$
Energy flux	50–500 MeV	3×10^{-10}	$\text{erg cm}^{-2} \text{ s}^{-1}$

Of the two specific targets of this observation, M87 is about 8° outside the error box, and is therefore clearly not the source of the detected γ rays. On the other hand, 3C273 lies well within the error box. Despite the large size of this error box, the following compelling arguments for an identification with 3C273, the second nearest of the powerful QSOs ($z = 0.158$), can be made. The probability of finding a known high-galactic-latitude X-ray source by chance within a $3^{\circ} \times 3^{\circ}$ error box is about 2%. However, the X-ray source 4U1226+02 ($\equiv 2A1225+022$) lies within the error box of CG291+65. This X-ray source has been positively identified with 3C273 (refs 3, 4). Also, it is stressed that the probability of finding a chance coincidence of the error box with a specific object, that is 3C273, is $<1\%$.

If this identification is correct the observed intensities at γ -ray energies can be compared with those at other wavelengths, as shown by the spectrum in Fig. 1. This shows that a major portion of the energy radiated by 3C273 is in the form of γ rays. Its γ -ray luminosity in the range 50–500 MeV is $\sim 2 \times 10^{46} \text{ erg s}^{-1}$ for $H_0 = 60 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

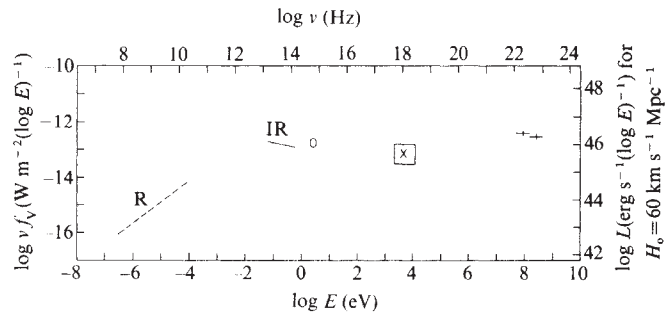


Fig. 1 Spectrum of 3C273 from radio to γ -ray energies. The intensity scale is given in units of power per decade. The radio (R), optical (O) and X-ray (X) data are those given in a compilation by Apparao *et al.*⁵. The IR data are from Rieke and Low⁶.

This has important consequences for the understanding of QSOs and warrants extensive efforts to further strengthen the identification. Repeated observations of 3C273 may yield signs of time variability, similar to those clearly established at other wavelengths, with amplitudes which tend to increase with increasing energy⁵. A second observation by COS B is planned for June/July 1978. Somewhat less directly, the identification could be strengthened by an extensive search for γ -ray emission of other QSOs. In this context, note that the newly discovered and nearest QSO, associated with 2S0241+622, lies within the error box of CG135+1 (ref. 5).

The intriguing fact that all γ -ray sources detected by COS B, including pulsars, unidentified galactic sources and possibly extragalactic sources, have a peak luminosity at γ -ray energies, may indicate some similarity in the emission process, or may reflect a selection caused by the limited dynamic range of detected γ -ray fluxes.

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Infrared observations of the most luminous quasar

The QSO Q 0420–388 is one of many discovered by Osmer and Smith in their objective prism survey of the southern sky, and one of ~ 10 now known with $z > 3$. Its brightness and large redshift indicate a luminosity at rest wavelength $1,475 \text{ Å}$ somewhat larger than any other measured. The source is particularly noteworthy because of the steepness of its observed visual spectrum over the range $3,600\text{--}6,800 \text{ Å}$, where $F_\nu \sim$

$\nu^{-2.1}$; this suggests that appreciably more power might be radiated in the infrared (IR). The near IR photometric observations reported here of Q 0420-388 ($z=3.12$) show that it is 13.5 ± 0.2 mag at $2.2 \mu\text{m}$. This observation and the optical observations of Osmer and Smith¹ indicate a bolometric luminosity $\geq 5 \times 10^{14} L_{\odot}$, for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$ confirming Q 0420-388 as the most luminous quasar for cosmologies with small q_0 .

Table 1 gives accurate positions for the quasar and a nearby star, as measured from the ESO B survey plates. These positions are derived from those of the five nearest SAO stars (194936, 194939, 194974, 194977, 194979) including the effects of the proper motions of these stars.

Using the 1.5 m telescope at CTIO and the InSb photometer of the Center for Astrophysics, we observed Q 0420-388 at $2.2 \mu\text{m}$ ($1.36 \times 10^{14} \text{ Hz}$) on 27 July 1977 and 30 July 1977, obtaining consistent results whose weighted mean is $K = 13.5 \pm 0.2$ mag, corresponding to a $2.2 \mu\text{m}$ flux of $2.5 \pm 0.5 \text{ mJy}$. We used a $35''$ diameter beam, and a beam separation of $96''$ in declination. The star in Table 1 is the only one visible on the Whiteoak extension of the Red Palomar Sky Survey that might interfere with our measurement of the quasar. This star is $34''$ SE, well outside our beam, and a separate measurement of its brightness gave $K = 12.9 \pm 0.7$ mag; the star is too faint and too far separated for scattered light from it to affect our measurement of the QSO.

The flux distribution $\lambda F_{\lambda} = \nu F_{\nu}$ is given in Fig. 1. Osmer and Smith¹ give a flux of 0.6 mJy at $6,077 \text{ \AA}$ (rest wavelength $1,475 \text{ \AA}$). Combined with our result, this gives $F_{\nu} \sim \nu^{-1.1 \pm 0.2}$ for the range $0.6-2.2 \mu\text{m}$. This spectral index is consistent with the range of values quoted by Osmer and Smith for the $0.36-0.68 \mu\text{m}$ region, and it may be that the spectral index of the quasar is -1.1 ± 0.2 over the entire $0.36-2.2 \mu\text{m}$ range. The high galactic latitude of the source, $|b^{\text{III}}| \sim 45^\circ$, suggests that the galactic extinction is low. Thus, a change in the apparent spectral index of the source would reflect its intrinsic behaviour. Q 0420-388 is not listed in the Parkes catalogue², so its flux at $2,700 \text{ MHz}$ cannot be more than 220 mJy . This result and our $2.2 \mu\text{m}$ measurement indicate that the spectral index between the radio and near IR is > -0.4 . From their observations of high redshift QSOs in the Molonglo survey, Baldwin *et al.*³ infer that there is a preponderance of such flat spectrum sources among high z objects.

We have estimated the bolometric flux using $\nu F_{\nu} \sim \nu^{-1}$ for $\lambda < 6,077 \text{ \AA}$, $\nu F_{\nu} \sim \nu^{-0.1}$ for $0.61 \mu\text{m} < \lambda < 2.2 \mu\text{m}$, and $\nu F_{\nu} \sim \nu$

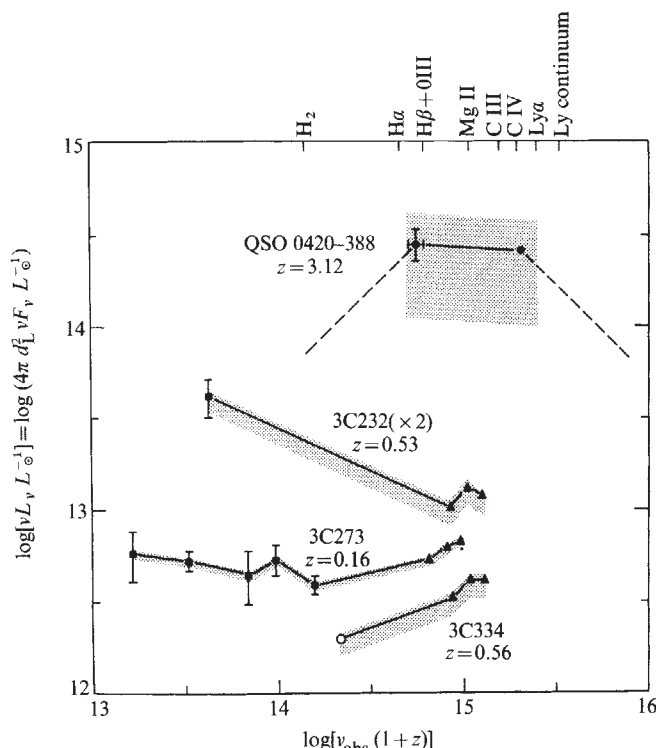


Fig. 1 Luminosity of Q 0420-388 and comparison quasi-stellar objects. The luminosity is expressed in solar units; the frequency is the rest frequency. The data points and connecting lines are drawn for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$. The edges of the shaded zones indicate the maximum luminosity, when $q_0 = 0$, and the luminosity when $q_0 = 0.5$. For Q 0420-388 the $\lambda_{\text{rest}} = 1,475 \text{ \AA}$ point is from Osmer and Smith¹; the datum at $\lambda_{\text{rest}} = 0.53 \mu\text{m}$ is from this paper. The dashed lines show the spectral behaviour assumed in calculating the luminosity. For the comparison quasars, $\blacktriangle$ are UVB observations from the compilation of Smith-Haenni⁷, and $\blacksquare$ are from Rieke and Low⁵. The $\lambda_{\text{rest}} = 1.41 \mu\text{m}$ point for QSO 3C334 is a $2.2 \mu\text{m}$ observation given by Oke *et al.*⁶.

Table 1 Precise positions

Object	$\alpha(1950)$				$\alpha(1950)$			
	h	m	s	s	°	'	"	"
Q 0420-388	04	20	30.07	± 0.05	-38	51	50.4	± 0.6
Star SE of QSO	04	20	31.95	± 0.05	-38	52	16.9	± 0.6

for $\lambda < 2.2 \mu\text{m}$. This gives $F_{\text{bol}} = (1.05 \pm 0.2) \times 10^{-14} \text{ W m}^{-2}$, or $L = 5 \times 10^{14} L_{\odot}$ if $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$. For $q_0 = 0$ or $q_0 = 1$ the values for L would be $7 \times 10^{14} L_{\odot}$ and $1 \times 10^{14} L_{\odot}$ respectively. More extensive IR observations would test the suitability of the spectral indices used above, and provide a better estimate of the luminosity. Our estimate will be too large if the broad bandpass $2.2 \mu\text{m}$ ($\Delta\lambda = 0.4 \mu\text{m}$) observation is strongly affected by H β and OIII, which are redshifted to just inside the bandpass. On the other hand, our luminosity estimate will be too small if the spectral distribution of Q 0420-388 continues its rise into the IR. If the spectrum rises as $\nu^{-1.1}$ out to $10 \mu\text{m}$, it will be 4 mJy (12.2 mag) at $3.5 \mu\text{m}$ and 13 mJy (8.7 mag) at $10 \mu\text{m}$, and the luminosity will be $8 \times 10^{14} L_{\odot}$. If Q 0420-388 rises into the IR as rapidly as 3C232, it will be $\sim 20 \text{ mJy}$ at $10 \mu\text{m}$ and the minimum total luminosity will be $13 \times 10^{14} L_{\odot}$. Detections at these low flux levels should be

possible with long integrations on large, low-background Southern Hemisphere telescopes.

The obvious sources for comparison with Q 0420-388 do have spectra that rise into the IR. The most luminous galaxy, Markarian 231, has a bolometric luminosity of $\sim 4 \times 10^{12} L_{\odot}$, and the most famous quasar, 3C273, has a bolometric luminosity of $\leq 3 \times 10^{13} L_{\odot}$ (ref. 4). The most luminous quasar observed by Rieke and Low⁵, 3C232, can be (generously) estimated to have a bolometric luminosity of $\sim 2 \times 10^{14} L_{\odot}$, assuming that at IR wavelengths longer than $10 \mu\text{m}$ it is similar to 3C273.

The luminosity of Q 0420-388 seems to be temporally sustained. From spectrophotometric and near IR studies of 28 QSOs, Oke *et al.*⁶ argued that a steep rise into the IR ($\alpha < -1.5$) might be a necessary but not sufficient condition for rapid variability of a QSO, and that if such variation occurred, the entire UV-near IR spectrum rose or fell. The visible-IR spectral index of Q 0420-388, $\alpha = -1.1 \pm 0.2$, is shallower than the $\alpha < -1.5$ cut-on value. Moreover, the persistence of Q 0420-388 on the Whiteoak extension, on the ESO B plate series, and on the discovery plates of Osmer and Smith suggests its relative constancy. A most important finding is that the quasar is evident on seven plates in the Harvard collection (ranging back to 1936) and there is no conclusive evidence for its having varied by more than $\sim 1/2 \text{ mag}$. Thus, the luminosity may be constant, at least for time scales of several years. Additional IR and optical observations to establish the temporal behaviour of Q 0420-388 are clearly important for defining the energy requirements of the source.

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Low energy neutrino elastic scattering and supernova explosions

COLGATE AND WHITE¹ have proposed that neutrinos could transport energy outwards during the gravitational collapse of a star, thus initiating a supernova explosion. Detailed calculations by Wilson² seemed to rule out this possibility, but Freedman³ pointed out in the context of unified gauge theories of weak and electromagnetic interactions that there could be both a large neutrino-neutron elastic scattering cross section and a very large coherent neutrino-heavy nucleus cross section. Wilson⁴ has recently recalculated the collapse of an iron core formed at the end of a stellar evolution from massive stars and shown that the calculations do simulate an explosion, and that a stronger explosion results from smaller coherent scattering off heavy nuclei. This surprising result arises because a smaller cross section allows more neutrinos to escape, lowering the pressure and thereby letting the neutron core reach a higher density before the outward shock wave is formed. We have calculated neutrino-nucleon and neutrino-heavy nucleus neutral current cross sections in weak SU(2)×U(1) and compared with those of the standard unified theory. Important differences are found which could make simulated supernova explosions easier to obtain. Possible experiments to test the two theories are outlined here.

The theory of neutral weak currents which agrees with all high energy neutrino experiments is the renormalisable and unified spontaneously broken gauge theory based on the group SU(2)×U(1) (ref. 5). This provided the basis for the recent calculations by Wilson⁴ and others^{6,7}. We have suggested previously an alternative theory⁸ based on the same group which we call weak SU(2)×U(1) (as it does not involve the photon) and which is very hard to distinguish from the Weinberg model⁵ in high energy neutrino scattering. Nevertheless, there are marked differences which should have a significant effect on the supernova calculations. In particular, in weak SU(2)×U(1) there is essentially no coherent scattering of neutrinos off heavy nuclei whereas the neutrino-neutron cross section is changed relatively little.

We shall now briefly derive these results, using the notation in ref. 8. In unified SU(2)×U(1), the neutral weak hadronic current is

$$J_\mu = A_\mu^3 + (1 - 2 \sin^2 \theta_w) V_\mu^3 - 2 \sin^2 \theta_w V_\mu^S \quad (1)$$

This gives the matrix elements for neutrino-nucleon scattering

at small momentum transfer

$$\langle n' | J_\mu | n \rangle = -\bar{u}_n' (\gamma_\mu + g_A^3 \gamma_\mu \gamma_5) u_n \quad (2a)$$

$$\langle p' | J_\mu | p \rangle = \bar{u}_p' [(1 - 4 \sin^2 \theta_w) \gamma_\mu + g_A^3 \gamma_\mu \gamma_5] u_p \quad (2b)$$

where $g_A^3 = -1.25$ is the isovector axial renormalisation factor. Experimentally $\sin^2 \theta_w \approx \frac{1}{4}$ and so equation (2b) predicts that the proton neutral current is nearly pure axial. For $\sin^2 \theta_w = \frac{1}{4}$ the nonrelativistic cross sections in the Weinberg model are then

$$\sigma(\nu n \rightarrow \nu n) = G^2 p^2 (1 + 3(g_A^3)^2) / 4\pi \quad (3a)$$

$$\sigma(\nu p \rightarrow \nu p) = 3G^2 p^2 (g_A^3)^2 / 4\pi \quad (3b)$$

where p is the neutrino momentum. (In refs 6 and 7 it was assumed that $g_A^3 = -1$, thus reducing the cross section (3a) by about 30%.)

For neutrino scattering off a nucleus with Z protons and N neutrons equation (1) gives

$$\begin{aligned} \langle (Z, N) | J_\mu | (Z, N) \rangle \\ = (1 - 4 \sin^2 \theta_w) Z - N + \text{noncoherent terms} \end{aligned} \quad (4)$$

This shows that the neutrino-nucleus cross section in the Weinberg model should have a large coherent part which is proportional to N^2 for $\sin^2 \theta_w = \frac{1}{4}$. Note that this coherent contribution in this model happens to be just the quantity Q_w that should have been measured as a parity violation in atomic bismuth⁹. It was the failure to observe this effect which led to alternative versions of the weak current.

In weak SU(2)×U(1) the vector and axial current are interchanged. Thus instead of equation (1)

$$J_\mu = V_\mu^3 + (1 - 2 \sin^2 \theta_w) A_\mu^3 - 2 \sin^2 \theta_w A_\mu^S \quad (5)$$

There is now no vector isoscalar component (in Freedman's³ notation $a_0 = 0$) and so instead of equation (4)

$$\langle (Z, N) | J_\mu | (Z, N) \rangle = Z - N + \text{non-coherent terms} \quad (6)$$

Instead of equations (2a) and (2b) we have

$$\langle n' | J_\mu | n \rangle = -\bar{u}_n' [\gamma_\mu + g_A^3 \gamma_\mu \gamma_5 (1 - 2(1 - \lambda) \sin^2 \theta_w)] u_n \quad (7a)$$

$$\langle p' | J_\mu | p \rangle = \bar{u}_p' [\gamma_\mu + g_A^3 \gamma_\mu \gamma_5 (1 - 2(1 + \lambda) \sin^2 \theta_w)] u_p \quad (7b)$$

where $\lambda = g_A^S / g_A^3$ and g_A^S is the isoscalar axial renormalisation factor. If axial charge is a good quantum number $g_A^S = g_A^3$; on the other hand the quark model¹⁰ gives $g_A^S = \frac{2}{3} g_A^3$.

In terms of the ratios R_n and R_p of νn and νp cross sections in weak SU(2)×U(1) compared with those of unified SU(2)×U(1) above in the nonrelativistic limit, we have for $\sin^2 \theta_w = \frac{1}{4}$, and $\lambda = 1$

$$R_n = 1, \quad R_p = 0.21 \quad (8a)$$

and for $\sin^2 \theta_w = \frac{1}{4}$, $\lambda = \frac{2}{3}$

$$R_n = 0.7 \quad R_p = 0.25 \quad (8b)$$

(We take the quantity $\kappa = m_\pi \cos \theta_w / m_w$ of ref. 8 to be approximately given by $\kappa = 1$.) Therefore from equations (6)–(8), in weak SU(2)×U(1), neutrino-neutron cross sections are relatively unaffected while neutrino-proton cross sections are reduced by a factor of 4 or 5 on account of a pure axial current being replaced by a nearly pure vector current. There is very little coherent scattering and then only for heavy nuclei with $Z \neq N$. Spin $\frac{1}{2}$ nuclei with $Z = N \pm 1$ scatter neutrinos like protons or neutrons respectively.

These predictions should change the character of the supernova calculations. The most important effects of neutrinos in the calculations based on the Weinberg model come from neutrino-electron, neutrino-neutron and neutrino-heavy nucleus collisions^{6,7}. Energy is transferred most effectively in νe^- scattering and this is unchanged (neglecting the electron mass) in weak SU(2)×U(1) (ref. 8). The neutrino opacity in the neutron core is also relatively unchanged—the neutrino may

have a slightly longer mean free path in the neutron core—but what is drastically changed is the ability of the neutrino to scatter from heavy nuclei. There is now a much smaller opacity gradient between the neutron core and the $A > 1$ shell and therefore neutrinos will be able to escape through the $A > 1$ shell much faster: this could make explosions easier to obtain as higher densities in the core will be possible.

Finally, we should point out that it would be of great importance to decide experimentally between the two types of theory. Freedman³ suggested that an experiment on coherent neutrino-heavy nucleus scattering was possible by detecting the recoil nucleus, using neutrinos in the 100–200 MeV range from a 'meson factory'. Certainly a large coherent cross section would clearly discriminate in favour of the Weinberg theory or one with similar structure.

An alternative possibility is suggested by equation (8). It should be possible to measure $\nu p \rightarrow \nu p$ in the non-relativistic region at a reactor such as ILL, with a good high intensity point source of neutrinos. Recoil protons could be detected in either gas or liquid targets. If the experiment is performed it should be relatively easy to determine from the size of the cross section whether the amplitude is predominantly Gamow–Teller, as it is in the Weinberg theory for $\sin^2 \theta_w = \frac{1}{4}$, or Fermi and, therefore, 4–5 times smaller ($3(g_A^2)^2 = 4.7$) as it is in weak $SU(2) \times U(1)$.

Note added in proof. Weak $SU(2) \times U(1)$ predicts an asymmetry in polarised electron–deuteron scattering which is smaller than that observed in the recent SLAC experiment¹¹. On the other hand, the standard model is not in agreement with the Oxford and Washington experiments in bismuth vapour⁹. This confused situation makes it even more essential to measure the elastic neutrino–proton and neutrino–heavy nucleus cross sections at low energies, so that a clean determination of the weak hadronic neutral current can be made in this energy range.

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Photofixation of carbon dioxide to formic acid *in vitro* using water as hydrogen source

THE function of photosynthesis *in vivo*, including subsequent dark reactions, is defined as the reduction of carbon dioxide coupled with oxidation of water to produce organic compounds. We now report the first example of a mimicking of the function of photosynthesis based on electron transport sensitisation^{1–4}. Although the product is not carbohydrate, we consider that the present reaction system consists of (1) electron transfer from the singlet excited state of an aromatic hydrocarbon such as pyrene (Py) or perylene (Pe) to an electron acceptor such as 1,4-dicyanobenzene (DCB) or 9,10-dicyanoanthracene (DCNA) in polar media; (2) successive electron transfer from the acceptor anion radical to carbon dioxide; (3) further reactions of carbon dioxide anion radical as demonstrated in

Table 1 Photochemical formation of formic acid sensitised by Pe–DCNA system

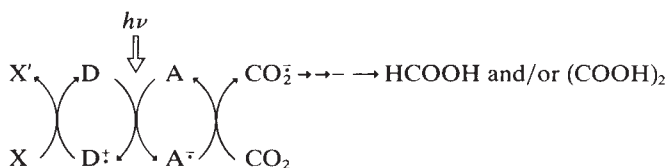
	Pe	DCNA (mg per 100 ml)	HCOOH	Yield (%)	
				vs Pe	vs DCNA
I	6.6	15.9	3.2	270	100
II	6.2	17.0	~0	~0	~0
III	5.5	15.0	5.9	590	195
IV	1.6	4.3	1.3	450*	150*

A solution containing Pe and DCNA was irradiated (wavelength > 313 nm, runs I, II, IV, panchromatic irradiation, run III) by a 300 W high pressure Hg-arc lamp under CO_2 gas bubbling for 8 h (runs I, II, III) or 20 h (run IV). Formic acid in the reaction mixture was converted to the butyl ester and determined by gas-chromatography (polyethylene glycol 20 M 53°C). Runs I, III, IV, $\text{CH}_3\text{CN}:\text{H}_2\text{O} = 95:5$; run II, absolute CH_3CN .

* The turnover numbers for Pe and DCNA were calculated as 6.5 and 12.8, respectively, from the decomposed amounts of the sensitizers during photoirradiation determined spectroscopically.

electrolytic reduction of carbon dioxide to formic acid and oxalic acid^{5,6}.

The reaction process will then be expressed schematically as follows:



Photochemistry of carbon dioxide in condensed phase has been little studied, and the first example was the reductive coupling of aromatic hydrocarbon anion radicals with carbon dioxide to give dihydroaromatic carboxylic acids⁷. If the anion radical is sterically hindered so that the C–C bond formation with carbon dioxide is unfavourable, we expect electron transfer from the anion radical to carbon dioxide to occur. Although we cannot confirm the energetic feasibility of the electron transfer process, the available redox properties of relevant species suggest that it is a good possibility ($E_{1/2}(\text{CO}_2) = -2.15$ V (ref. 5), $E_{1/2}(\text{DCB}) = -2.00$ (ref. 8) ~ -2.48 V (ref. 9). There are no available directly comparable data for DCNA; however, the more negative value of $E(\text{A}/\text{A}^-)$ for DCNA (-0.82 V (ref. 10)) than that for DCB (-0.65 V (ref. 11)) would indicate the stronger reducing power of DCNA⁸. Furthermore, the reductive fixation of CO_2 *in vivo* is coupled with the oxidation of NADPH which is apparently a weaker reducing agent than DCB⁸ ($E(\text{NADP}/\text{NADPH}) = -0.32$ V (ref. 12)). Thus, we carried out photoinduced electron transfer reactions in the atmosphere of carbon dioxide. The results are presented in Table 1 and 2.

The important results are summarised as follows. (1) An aqueous polar solvent is the essential requirement for the reaction. When dry acetonitrile was used, formic acid was scarcely produced (Table 1, run II, Table 2, run III). Furthermore, the yield of formic acid increased with the concentration of water (Table 2, runs, I, II). (2) As a matter of course, carbon dioxide is indispensable as the carbon source of formic acid (Table 2, run VI). (3) Qualitative confirmation of formic acid formation in other solvents such as aqueous methanol and aqueous DMF by means of spot tests¹³ indicated that these organic solvents did not play an essential part in reducing carbon dioxide. The role of organic solvents is assumed to dissolve D and A. (4) The D–A pair was indeed recycled, as evidenced by a yield of formic acid higher than 100% based on the initial concentration of D and A (Table 1, runs, I, III, IV). To recycle the electron transport system, another reducing species (X in the reaction scheme above) is required. We cannot yet draw any conclusion on the nature of X by which D is reproduced from D⁺, but probable candidates are OH^- , HCO_3^- and/or HCOOH . Iodometry of the reaction mixture (Py–DCB–TPA in $\text{CH}_3\text{CN}:\text{H}_2\text{O} = 5:1$, panchromatic irradiation for 8 h under a CO_2 pressure of

Table 2 Photochemical formation of formic acid sensitised by Py-DCB-TPA system

	[Py]	[DCB] ($\times 10^{-2}$ mol l $^{-1}$)	[TPA]	[HCOOH] ($\times 10^{-4}$ mol l $^{-1}$)
I	1.9	5.4	5.2	5.2
II	1.7	6.0	4.5	17
III	1.8	5.4	5.7	~0
IV	1.9	—	6.0	9.6
V	1.8	5.8	—	8.6
VI	1.9	5.6	5.4	0

A pressure tight pyrex vessel containing a solution of sensitisers was pressurised (4 kg cm $^{-2}$) with CO $_2$ (runs I–V) (or N $_2$) (run VI) and irradiated by a 300 W high pressure Hg-arc lamp for 8 h. Formic acid in the reaction mixture was esterified to the benzyl ester and determined gas-chromatographically. Runs I, VI, CH $_3$ CN:H $_2$ O = 95:5; runs II, IV, V, CH $_3$ CN:H $_2$ O = 5:1; run III, absolute CH $_3$ CN.

5 kg cm $^{-2}$) confirmed the formation of peroxide, suggesting that D $^+$ was reduced to D at least in part by the reaction, OH $^- \rightarrow 1/2$ H $_2$ O $_2$. (5) The turnover numbers of sensitiser (2X[HCOOH]/ Δ [sensitiser]) were determined to be 6.5 for DCNA and 12.8 for Pe (Table 1, run IV).

We have not yet determined the quantum yield of formic acid production, as the kinetics of the total reaction is complicated by several possible secondary reactions. A very coarse estimation of apparent efficiency could, however, be made by dividing the product by the energy input. The result shows that $\sim 3 \times 10^2$ mol of photon is required to reduce 1 mol of CO $_2$. This efficiency is extremely poor compared with the natural realisation of light conversion by photosynthesis. The possible reasons are: (1) antenna pigments are absent so that the cross-section of photoabsorption is small. (2) As the oxidised and the reduced products exist together in a homogeneous solution, back-reactions such as D $^+$ + A $^- \rightarrow$ D + A, CO $_2^-$ + D $^+ \rightarrow$ CO $_2$ + D and others will come into effect. (3) The product is not stable and reactions such as HCOOH $\rightarrow$ CO + H $_2$ O or CO $_2$ + H $_2$, HCOOH + D $^+ \rightarrow$ H $^+$ + 1/2 H $_2$ + CO $_2$ would reduce the apparent yield 14 . Separation of oxidising and reducing compartments by electron transport membrane would be a promising strategy to refine the present mimicked photosynthesis.

In conclusion, the results in Tables 1 and 2 indicate that the main overall reaction is the photoreduction of carbon dioxide with water as follows:



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Io–U dating of the Ouljian stage from Torre Garcia (southern Spain)

IN this note we discuss the reliability of the Io–U dating method and report on the age of fossils from the Ouljian stage, outcropping along the Mediterranean coast in southern Spain. That this method can produce good results is evident from the fact that the measured ages for these fossils, all from the same stratigraphic horizon, show a standard deviation of only 6,100 yr, which is well within our precision limits. The mean age of the samples is 98,000 yr.

The dating of mollusc shells by the Io–U method has been controversial; some of the ages obtained in this way have been incompatible with other age data, and some samples from the same epoch have shown a considerable scatter in their ages. It seemed that the ‘closed system’ condition, a requirement for successful application of this method, is frequently violated. However, certain indications, such as the absence of ^{232}Th , and $^{234}\text{U}/^{238}\text{U}$ ratios compatible with the calculated Io–U ages suggest that little or no contamination occurred. Szabo and Rosholt 1 have proposed a model to correct for the suspected open character of the system, this model has been criticised by Kaufman *et al.* 2 and it seems to be of only limited interest for this study.

Although the value of the Io–U dating method is in doubt, it is the only technique available for dating events occurring between 30,000 and 250,000 yr ago. In order to test the reliability of the Io–U method we have analysed six mollusc shells which are contemporaneous—that is, they come from the same stratigraphic horizon, the Ouljian stage. The best way to check the reliability of the method is to see if the measured ages show a significant dispersion and if the calculated absolute ages are reasonable. The rocks of the Ouljian stage outcrop in Spain and North Africa; it is important to establish an absolute age for this stage to study the secular changes in the level of the sea during the Quaternary. Because the outcrops of the Ouljian at both sides of the sea of Alboran occur at different elevations, dating of this stratigraphic level provides information about recent tectonic movements in this region.

We report here on six shells from the Ouljian, outcropping in the region of Torre Garcia along the beach of the Gulf of Almeria. The Pleistocene geology of this region has been discussed by Overejo *et al.* 3 . Sandstones of the Ouljian, containing many *Strombus bubonius* and *Pectunculus* outcrop at a few points at 5 m above sea level; most of this formation is covered by recent dune sand. A unique level characterised by *Strombus* has been recognised $^{4-6}$. This level has been correlated with the Ouljian stage in Morocco 4 which was dated between 80,000 and 120,000 yr (refs 4, 7). One age of 80,000 yr has been published 7 for a *Pectunculus* shell coming from the *Strombus* episode about 50 km NE from our sampling location at Torre Garcia. The six samples we analysed from the same horizon were cleaned mechanically; we removed about 1 mm of the superficial part. We worked mainly on the most massive parts of the shells of the *Strombus*, but for two shells, we have also analysed the thin section. All the samples were in good condition except samples I and III which had been attacked by marine organisms in some parts.

α spectrometry was used to measure uranium and thorium isotope abundances. Using the method of Goldberg *et al.* 8 , these elements in the dissolved samples were chemically separated by coprecipitation, followed by purification with organic solvents and electrodepositions. ^{232}U and ^{234}Th (a β emitter), were used as tracers. Results are shown in Table 1.

The ^{232}Th concentration was <0.001 p.p.m. in all samples; the ^{232}Th activity for the total sample (1–4 g) is barely resolvable above the background noise. It is also clear that, after the observed ^{228}Th signal has been corrected for background noise

Table 1 Analysis of six mollusc shells from the Ouljian stage

Sample	$^{234}\text{U}/^{238}\text{U}$	U (p.p.m.)	^{234}U (d.p.m. g $^{-1}$)	$\text{I}_0(^{230}\text{Th})$ (d.p.m. g $^{-1}$)	Age (yr)	% Aragonite
I*	1.08 $\pm$ 0.04	0.607	0.491	0.286	93,000	>90
I†	1.12 $\pm$ 0.06	0.789	0.659	0.420	108,000	>90
II	1.075 $\pm$ 0.04	0.514	0.412	0.253	102,000	Argonite > calcite
III	1.09 $\pm$ 0.04	0.846	0.689	0.414	98,000	Argonite < calcite
IV	1.09 $\pm$ 0.04	0.843	0.686	0.403	95,000	100
V*	1.12 $\pm$ 0.03	0.662	0.553	0.306	88,000	>90
V†	1.13 $\pm$ 0.04	1.03	0.875	0.534	101,000	>90
VI	1.08 $\pm$ 0.05	0.469	0.378	0.229	99,000	100

From the age values shown the mean is 98,000 yr $\pm$ 6,100 yr.

* From massive part of the shell.

† From thin part of the shell.

and the ^{228}Th introduced via the tracer ^{232}U , there is no ^{228}Th in our samples. Of course, this is to be expected, because ^{228}Th is a radiogenic daughter of ^{232}Th . However, ^{228}Ra (period 6.74 yr), occurs in this decay chain, and in certain situations this element is very mobile and thus the absence of ^{228}Th suggests that no recent contamination has taken place at least by radium. Uranium concentrations range from 0.6 to 1.2 p.p.m. In shells I and V we have analysed the thin parts where the U concentration is significantly greater than in the remainder of the shell. The ratios $^{234}\text{U}/^{238}\text{U}$ are always less than 1.15 which corresponds to the value in present-day seawater. The measured $^{234}\text{U}/^{238}\text{U}$ ratios are compatible with the calculated ages for the samples to within our precision limits. The ratios in the thin parts of the shells I and V are somewhat larger than for the massive samples. The greater part of each sample is aragonitic, with calcite content less than 10%; two samples (II, IV) contain more than 10%.

The uranium concentration in the shells of living molluscs is very low, often less than 0.1 p.p.m. but in the fossil shells it can be several p.p.m. The concentrations we have measured are not very high but uranium enrichment has obviously taken place. This occurs very rapidly after the animal has died⁹: Kaufman *et al.*² have confirmed this conclusion by a statistical analysis of the uranium concentration in mollusc shells of known ages. The uranium enrichment in the shells of molluscs which have just died seems to be related to oxidation–reduction reactions occurring during the decomposition of organic material of the mollusc body. Blanchard *et al.*¹⁰ have also shown that the superficial parts of the shell are selectively enriched—conforming to our observations.

This post-mortem enrichment in uranium may cause errors of a few thousand years in dating and is thus of no great importance for our purpose. The samples may be affected by other contamination events at later stages, however. But in the present case we have no clear indication of this. For instance, contact with freshwater could cause changes in the $^{234}\text{U}/^{238}\text{U}$ ratio and in the ^{232}Th abundance. Our samples (V, I) show that the uranium enrichment of the thin parts of the shells is associated with an increase of the I_0 activity. No ^{232}Th activity was detected, however, so the increased I_0 activity is probably due to *in situ* production. The calculated ages of the thin parts of the shells tend to be somewhat greater than the age of the massive parts; because I_0 is not very mobile these age differences could be caused by an uranium loss occurring mainly in the superficial part. The $^{234}\text{U}/^{238}\text{U}$ ratios of all the samples are (within error limits) compatible with the I_0 –U ages and hence continental water uranium contamination with its much larger $^{234}\text{U}/^{238}\text{U}$ ratio is not likely. It is also obvious that the calcite content of the shells does not show any correlation with the ages. Calcitic samples, which often contain less uranium (this is not the case here) give ages very similar to those for aragonitic samples².

Ignoring the 108,000 yr age for the thin part of shell I, which could be the result of a small loss of uranium from the outer part,

we note that the ages obtained from the remaining seven samples range from 88,000 to 102,000 yr. The mean age of these seven samples is 97,000 yr with a standard deviation of 4,900 yr. Our experimental precision is about 7%, so our results are well within precision limits. Including the 108,000 yr age, the mean age would only increase to 98,000 yr with a standard deviation of 6,100 yr. These results are in general agreement with previously obtained ages for the Ouljian⁷ but show far less scatter.

These results indicate implicitly that 98,000 yr ago the sea level was about 5 m higher, relative to the present location of the Ouljian outcrop from which our samples came. This is in contrast to sea level investigations which have indicated that the worldwide palaeo-sea level was 10 to 20 m below the present level at about 100,000 yr ago. The accuracy of our work rules out the possibility that the true age of our samples is $125,000 \pm 10,000$ yr, an age at which sea level was higher than today. We interpret our results to mean that the coast of the Gulf of Almeria has experienced a net upwards vertical movement of 5 m with respect to sea level and a net total uplift of 15–25 m relative to a fixed reference frame, since 98,000 yr ago, that is, 15–25 cm per thousand years.

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Dissipation of tidal energy by Antarctic ice shelves

FLEXING of ice shelves may dissipate enough tidal energy to have a significant effect on the Moon's retreat from the Earth. It is generally agreed that tidal friction slows the rotation of the Earth and decelerates the Moon in its orbit¹. The total energy dissipated can be calculated from observations of the Moon's motion and agrees closely with estimates of the gravitational work done by the Sun and Moon on the ocean tidal bulge². Therefore dissipation of tidal energy is considered to occur mainly in the ocean with small upper limits being set on energy losses in the Earth's mantle and core. Because the amount of tidal dissipation in shallow seas³ and internal tides⁴ is uncertain it is of interest to search for additional dissipative mechanisms. In this note the possibility of tidal bending of ice shelves is examined.

An ice shelf is an ice sheet floating on the sea. In Antarctica ice shelves cover an area of $>1.5 \times 10^6 \text{ km}^2$. Thicknesses range from 100 m at the seaward ice front to $>1,800 \text{ m}$ at the grounding line where the ice is attached to land⁵. Ice shelves rise and fall freely with the tide except in a bending zone near the grounding line where the ice shelf deforms. A discussion of different types of grounding line and their expected dynamic behaviour has been given by Hughes⁶. An estimate of the rate at which energy is dissipated in the bending zone can be made by assuming that ice is deforming plastically. A transient creep process with parabolic strain hardening is thought to be appropriate for stress rates occurring in tidal flexure^{6,7}. Using equations derived by Holdsworth⁷ for the surface bending stress σ , average strain rate $\dot{\epsilon}$, and exponential damping factor λ ($=\lambda p/2$ in Holdsworth's notation), the following expression can be derived for the rate at which energy is dissipated;

$$\dot{E} = \frac{2hL\sigma\dot{\epsilon}}{\lambda} = 1.24 \times 10^7 h^{29/30} \xi^{1/2} \dot{\epsilon}^{16/15} L \text{ watts}$$

where $2h$ is the ice thickness at the grounding line, L the total length of hinge line and ξ the tidal amplitude. This simply assumes that energy is dissipated at the rate of $\sigma\dot{\epsilon}$ per unit volume and that the total volume of ice affected is $2hL/\lambda$. Taking values for the Antarctic of $2h$ as 500 m, L as $16 \times 10^3 \text{ km}$ (ref. 8) and a semi-diurnal tidal amplitude ξ as 1 m gives $\dot{E}$ as $2.0 \times 10^{12} \text{ W}$.

There are many uncertainties associated with this figure. A value for the maximum surface bending stress has been used as though it applied throughout the whole flexing region; however, on the assumption that the bending strain decays linearly towards the neutral axis the stress will decay parabolically⁷ reducing the energy dissipated by a third. The various types of grounding line are expected to have different stress patterns, but as the calculation here is for the case of least average tidal flexure strain⁶ the total dissipation is probably an underestimate. Crevasseing on the top and bottom may reduce the dissipation considerably. The length of the grounding line L includes both ice shelves and outlet glaciers and was derived⁸ from maps published up to 1965. Perhaps the greatest change to the mapped coastline of Antarctica since then has occurred on the Ronne Ice Shelf⁵. However, although the position of the coast has been altered, there has been little change in the actual length of coast. The greatest error probably lies in not considering ice rise boundaries, and thus the figure used should be regarded as a lower bound. There is more uncertainty in the value used for the mean thickness of ice at the hinge zone. Although the major ice shelves have at least some depth soundings, data for grounding line regions are sparse. Using published ice thickness contour maps for the Ross Ice Shelf⁹ and the Ronne Ice Shelf⁵ (excluding the Filchner Ice Shelf), their mean thickness at the grounding line can be calculated as 610 m and 990 m respectively. These

two ice shelves contain a quarter of the total length of grounding line. Another approach is to take the mean thickness of all Antarctic ice shelves, 390 m (ref. 8), so that by assuming a value for the mean thickness at the ice front where the ice is thinnest the mean grounding line thickness can be estimated. Taking a value at the ice front of 200 m gives the mean thickness at the grounding line to be 580 m. So again, the actual figure of 500 m used in the calculation probably errs on the side of caution. The dissipation rate of $2.0 \times 10^{12} \text{ W}$ is unlikely to be underestimated because of errors in h and L . A reasonable error, considering the competing nature of all the uncertainties, might be of the order of 50%.

One way of checking whether this amount of dissipation by bending is feasible would be to estimate the total tidal energy flux under ice shelves. In general, the energy flux per unit area perpendicular to the direction of propagation is given by $\frac{1}{2}\rho g u \xi \cos \theta$ where ξ and u are the amplitudes of tidal height and current respectively. For a progressive wave $\theta = 0$ and the maximum current flow inwards occurs at high tide; for a standing wave $\theta = \pi/2$ and there is no net energy flux. Again, because of the almost complete lack of relevant data for Antarctica it is impossible to perform a meaningful calculation. However, if the energy flux under a section of the Filchner Ice Shelf, where a value of tidal current of 0.1 m s^{-1} is assumed¹⁰ for a site where the depth of the water column under the ice front is 600 m (ref. 11) is taken as typical for the rest of Antarctica, then for $\theta = 0$ the required energy flux would be provided by only $6.6 \times 10^3 \text{ km}$ of ice front. It is therefore reasonable to say that there is probably enough incoming energy to allow the dissipation rate calculated for tidal bending.

To account for the astronomically observed lunar acceleration a dissipation rate of $5.7 \pm 0.5 \times 10^{12} \text{ W}$ is required². This is close to the estimate of $5.0 \pm 0.3 \times 10^{12} \text{ W}$ for the work done on the ocean surface by the luni-solar torque². The problem is to determine how the dissipation occurs in the ocean and to partition the energy loss among the mechanisms. An estimate of the frictional losses in shallow seas and continental shelves³ is $1.5 \pm 0.8 \times 10^{12} \text{ W}$. However, many of the areas where dissipation occurs are situated in high latitudes where the main tidal torque is reduced by a factor $\cos^2 \psi$ where ψ is the latitude¹². The same problem applies to ice shelf dissipation. It is uncertain how dissipation in high latitudes and in localised regions can affect the rest of the ocean tide. A more even dissipation of tidal energy would be by internal (baroclinic) tides, but an estimate of the energy loss is of the order of 10% of the total tidal dissipation⁴. After allowing for errors the total dissipation from these three mechanisms can therefore barely account for the required amount.

If tidal energy is being lost under ice shelves then one might expect to find that the age of the semi-diurnal tide is high. The age of the tide is the time interval between the time of new or full Moon and the time of the spring tide. Other areas of the ocean where the age of the tide is greater than 60 h are associated with strong tidal dissipation¹³. There are few reliable tidal records from Antarctica but the majority show ages greater than 60 h. The most detailed analysis has been carried out by Cartwright¹⁴ on records gathered along the west coast of the Antarctic Peninsula for observation periods of up to 9 yr. The results clearly show increasing age of tide with latitude, culminating in ages of 165 h for tidal records obtained on George VI Ice Shelf. Gravimetric readings on the Ross Ice Shelf also show a very high age of the semi-diurnal tide but with small amplitudes¹⁵. Conversely, the lowest age found in Antarctica (17 h) has been measured on the edge of the Filchner Ice Shelf¹⁶. This may be an indication of the complexity of the interaction between ice shelves and tides especially near critical latitudes where tidal and inertial frequencies are equal¹⁴. In principle the energy flux under an ice shelf could be calculated from simultaneous measurements of tidal amplitude and current. It would then be possible to decide the importance of tidal dissipation both by bending and by frictional losses along the bottom of the ice shelf and the bottom of the sea.

If ice shelves form a major sink for tidal energy then there are implications on a geological time scale. It might be expected that the times of maximum slowing of the Earth's rotation would coincide with times of maximum extent of ice shelves. Within the constraints imposed by the lack of data, this seems to be the case; extensive glaciation occurs in the periods preceding 'turning points' when the Earth increases its spin velocity¹⁷.

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Melting of Antarctic icebergs

PROPOSALS for supplying water to arid regions through the towing and utilisation of Antarctic icebergs^{1–5} have generated considerable interest. Simulation studies on the towing of unprotected icebergs to southern continents⁶ suggest that the towing distance, ocean currents and the iceberg deterioration rate are of major importance. The distance and current components may be optimised by a careful choice of iceberg position, although much further study of currents in southern latitudes is required. The rate of deterioration is probably more difficult to calculate as a number of melting and calving mechanisms may be operating. In this note some initial studies of iceberg melting are reported and some of the problems involved in further investigations are considered.

Above-sea surface melting is probably due to solar radiation and wind-enhanced convection. Under-sea melting may occur through turbulent natural convection. This should be increased by iceberg surface discontinuities, iceberg wallowing or by release of entrapped air bubbles on melting. Calving could result from side undercutting by wave action⁷, differential melting along cracks and low density areas⁸, stress on underwater shelves formed through layered melting⁹ or thermal stress-induced disintegration following calving in warm water⁶.

If under-sea melting is an important ablation mechanism, then one or more of the following three effects might be detectable. (1) The lighter freshwater could rise up the sides of the iceberg with little mixing to produce a pool of less saline water. If this happens then it would be possible for meltwater to be collected in warm waters by building a shallow pen around the iceberg. (2) The lighter freshwater may entrain warmer, saltier water from the surrounding sea and thus be responsible for considerable vertical transport of deep water and nutrients¹⁰. (3) Melting could occur in a stratified manner and the freshwater may then disseminate in layers. This type of melting has been detected in model studies⁹.

Thus evidence of gross salinity variation near an iceberg would support mechanism (1), and a temperature rise would support mechanism (2). To test for these two mechanisms,

salinities were measured around three icebergs in the northern Ross Sea/Southern Ocean. (Mechanism (3), suggested only recently⁹, was not tested. The salinity changes involved would probably not be detectable with the small portable salinometer used.)

All measurements were made from the 18-m yacht Solo using a Yellow Springs Instruments portable salinity/temperature meter (sensitivity, $S = \pm 0.05\%$), equipped with 200 m of cable. Salinities were recorded in the open sea in the following conditions. (1) Motoring as close as safety permitted (20 m from underwater ram) on the lee side of an iceberg; as the yacht drifted slowly downwind, measurements were taken continuously. (2) Circling an iceberg (speed ~ 2 knots) at known distances (closest weather side measurements were at 60 m). The meter was standardised at ambient temperature before use with a standard seawater solution¹¹. Salinity data were gathered at distances of 20 m to 800 m (range finder values) and at depths of 5, 10, 25, 60, 100 and 200 m. A correction for drag on the weighted probe was not applied but depths are probably overestimated by no more than 20%.

The results are shown in Table 1. No variation of salinity with distance from icebergs was found. A temperature inversion at a depth of 50–60 m around iceberg no. 3 was recorded but this extended for at least 2 km. Again, no salinity variation with distance was detectable along this discontinuity.

It is perhaps not surprising that there was no evidence of a salinity gradient with distance around icebergs, even in water to $+5.6^\circ\text{C}$. It seems likely that naturally positioned icebergs are not melting rapidly or that if melting is substantial then either mixing or rapid layered spreading may occur. If only little melting is occurring this might be because of the formation of a narrow insulatory envelope of cold water. Disturbance of such an interface by iceberg towing would then increase the melting rate dramatically. Unambiguous results will probably only be achieved by using a more accurate meter very close to an iceberg (possibly a dangerous undertaking).

No salinity step structure like that observed in the Weddell Sea¹² was observed, but a more accurate meter might detect such salinity steps in this region of ocean. However, a gross thermal inversion at 60 m was observed near iceberg 3. This thermal inversion is large for waters south of the Antarctic Convergence. Similar salinity profiles without step structure have been maintained in tanks with sloping boundaries¹³. Rapid changes in surface sea temperature at the Antarctic Convergence were measured further north at 59°S , 163°E . The lack of a shallow warm surface layer in the regions studied may be due to consistently overcast, cold, foggy weather.

Table 1 Summary of observations

	Iceberg 1	Iceberg 2	Iceberg 3
Date	16/1/78	17/1/78	29/1/78
Size*	0.5 km $\times$ 0.2 km $\times$ 30 m	0.4 km $\times$ 0.3 km $\times$ 30 m	1.2 km $\times$ 0.8 km $\times$ 35 m
Latitude $^\circ\text{S}^\dagger$	66.8	66.8	65.3
Longitude $^\circ\text{E}^\dagger$	173.4	173.5	164.7
Sea temp. ($^\circ\text{C}$)	$-1.4\ddagger$	$-1.4\ddagger$	$+2.4\S$
Sea temp. ($^\circ\text{C}$)			$+5.6\ $
Air temp. ($^\circ\text{C}$)	-1.7	-1.8	$+1.0$
Swell height (m)	1.5	1.5	3
Swell direction	270°	270°	320°
Swell period (s)	7	7	6
Wind speed (m s^{-1})	2.5	4.5	7.5
Wind direction	270°	270°	320°
Cloud	7/8	5/8	8/8
Salinity $\% \P$	34.5 $\ddagger$	34.5 $\ddagger$	34.8 $\S$ 35.1 $\ $

* Above sea.

† Celestial navigation.

‡ All depths and distances.

$\S$ 5–25 m depths, all distances.

$\|$ 60–200 m depth, all distances.

$\P$ Measurements made to $S = \pm 0.05\%$

Calving is probably the most important ablation mechanism in both northern⁷ and southern waters. On Solo's voyage from Sydney to Cape Adare and back, 244 icebergs were logged, most of which had freshly cleaved surfaces and exhibited little rounding. However, many rounded 'bergy bits' and 'growlers' were encountered downwind from most large icebergs.

It seems probable that data on the deterioration of icebergs relevant to towing proposals will only be gathered by prolonged study and by towing a test iceberg across the Antarctic Convergence.

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Absolute age of the base of the hominid-bearing beds in Eastern Java

THIS note presents preliminary data on the absolute age of the base of the hominid-bearing beds in Eastern Java. We approached the problem in two steps. First by comparing the stratigraphic sequence of Eastern Java with that of Olduvai Gorge using published data on radiometric dates, magnetic polarity stratigraphy, and the hominid fossil record (Fig. 1). And second, by analysing the planktonic diatom assemblages from the marine intercalations in the lowermost hominid-bearing beds and from the underlying marine sediments from Eastern Java (Fig. 2). The stratigraphy of Olduvai Gorge (Fig. 1) is compiled from various sources¹⁻⁶. The base of the Gorge is represented by metamorphic rocks⁶, overlain by ignimbrites whose uppermost part has been K-Ar dated at 2 Myr (refs 5, 6). The sediment sequence from Bed I to the top of the Gorge is represented by volcanogenic, lacustrine and fluvial deposits. The earliest hominid fossils and stone tools occur in the upper part of Bed I, at about 1.8 Myr (refs 1, 4, 5).

The Javanese fossils of early man were found only in the Solo Zone between Surakarta, on the border between Central and Eastern Java, and Modjokerto in Eastern Java⁷. The type section with hominid beds is Sangiran dome, located to the north of Surakarta. Data for the construction of the lithological sequence of the Sangiran dome were taken from Van Bemmel⁸. Marine marls and limestones are conformably overlain by 200 m of Putjangan Beds. The basal part of the Putjangan Beds is composed of volcanic breccias containing marine and freshwater molluscs. The rest of the Putjangan Beds is composed of black clays of lacustrine origin, with volcanogenic material. An intercalation of marine clay, about 0.5 m thick occurs in the lower part of the black clay bed and probably originated in a eustatic sea level change. The Putjangan Beds are conformably overlain by 45 m of fluvialite Kabuh Beds which, in turn, are separated from the overlying Notopuro Breccias by a

sharp disconformity. Tektites have been found on the erosional surface separating the Kabuh Beds and Notopuro Breccias^{8,9}, and have been dated by K-Ar and fission track methods at 0.7 Myr (refs 10, 11). A microtektite layer identified in a number of deep-sea cores from the Indian and Pacific Oceans occurs at the Brunhes-Matuyama boundary (Fig. 1)¹². The tektites and deep-sea microtektites are identical¹³, and differ from volcanic glass shards¹⁴ and Indonesian lavas¹⁵. Tektites were also found

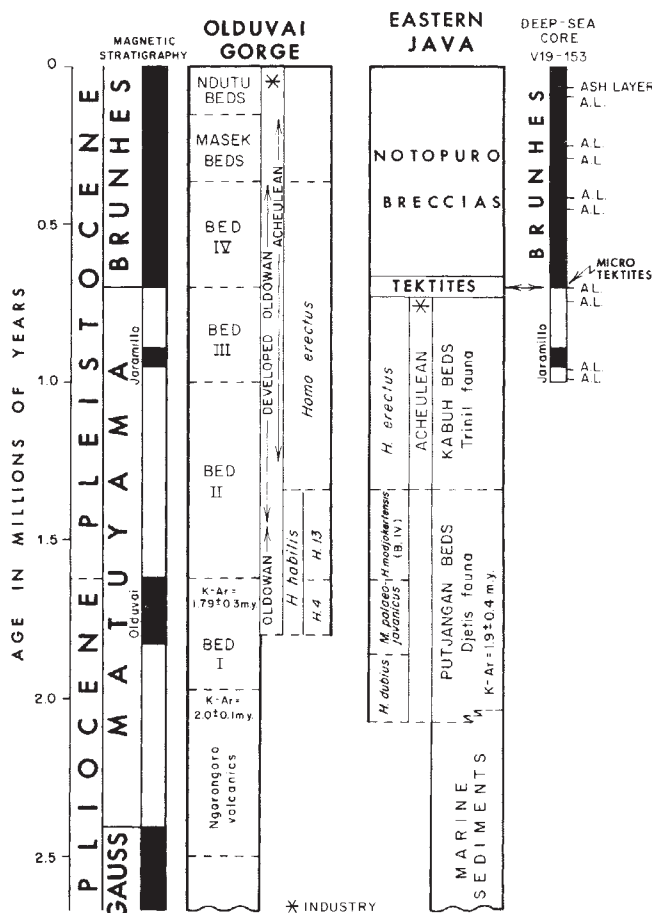


Fig. 1 Stratigraphy of hominid beds of Olduvai Gorge and Eastern Java, and correlation of hominid fossils compiled from various sources.

redeposited in younger sediments of Java and thus their stratigraphic value has been contested. However, this is not applicable for Sangiran since it is unlikely that tektites up to 10 cm long were redeposited upwards and concentrated on top of the Kabuh Beds in the dome-like structure. They are, therefore, a good stratigraphic marker for the Brunhes-Matuyama boundary of Sangiran.

Pliocene/Pleistocene mammalian faunas of Eastern Java are divided in three groups¹⁶: Djetis, associated with the Putjangan Beds, Trinil from the Kabuh Beds, and Ngandong from Late Pleistocene sediments. Hominid fossils found in Eastern Java are associated with all three mammalian faunas. The most abundant hominid fossils were recovered from the Kabuh Beds. This may be attributed, in part, to the composition of these fluvialite beds, which are more easily eroded than the clays of the Putjangan Beds. At Modjokerto only one skull (*Homo modjokertensis*) has been recovered from the Putjangan Beds¹⁷, and its original position in the beds has not been contested¹⁸. In Sangiran dome, however, fossils from the Kabuh Beds were often carried downslope to the level of the Putjangan Beds due to erosion¹⁹. Only a few hominid fossils of Sangiran (Fig. 1),

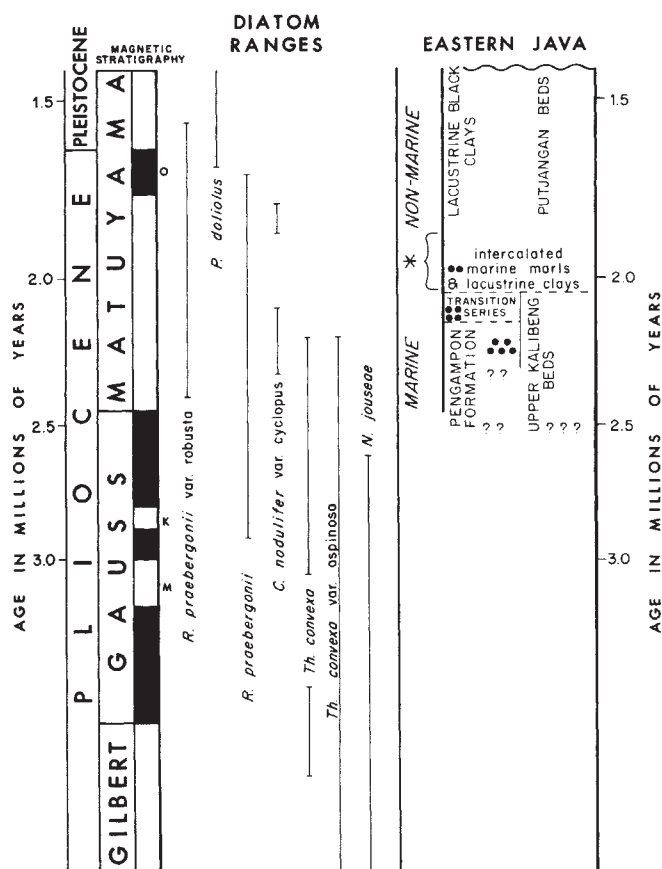


Fig. 2 Proposed correlation between deep-sea piston core V28-179 and the marine/non-marine transition in Eastern Java. The asterisk indicates the probable margin of error in dating this transition. Closed circles represent the number of samples examined in each formation.

however, have been attributed to the Putjangan Beds with reasonable certainty¹⁹⁻²¹.

Weidenreich²² studied the Javanese hominid fossils and suggested the following evolutionary trend, based on their morphological characteristics: *Meganthropus palaeojavanicus* → *H. modjokertensis* (which he named *robustus*) → *H. erectus*. Some hominid fossils from Late Pleistocene sediments, associated with the Ngandong mammalian fauna, correspond to an advanced *H. erectus*, or the *Neanderthal*²³. Tobias and Von Koenigswald²⁴ compared hominid fossils attributed to the Putjangan Beds with fossils from Olduvai Gorge. They concluded that *H. modjokertensis* (IV and B) is similar to *H. habilis* (Hominid 13) from the lower part of the Olduvai Bed II, while *M. palaeojavanicus* is similar, but a somewhat more primitive form than *H. habilis* (Hominid 4) from Olduvai Bed I (Fig. 1). This correlation is in agreement with Weidenreich's²² evolutionary trend of the Javanese hominids. *H. dubius*^{20,21} may represent, according to Von Koenigswald²¹, the earliest form of hominids recovered from Java. A K-Ar age of 1.9 ± 0.4 Myr has been obtained for the Putjangan Beds near Modjokerto, a few metres below the level where the skull of *H. modjokertensis* was recovered^{25,26}. Thus, the available data compiled in Fig. 1 suggest that the boundary between the Kabuh and Putjangan Beds may be tentatively placed about the middle of Bed II of Olduvai Gorge, and that the base of the Putjangan Beds in Eastern Java is older than the 1.8 Myr fossiliferous level of the Olduvai Gorge Bed I.

Marine diatoms are present in the marine sediments of the Upper Kalibeng Beds of Eastern Java (Fig. 2). In the basal part of the overlying Putjangan Beds, marine sediments (containing diatoms) are intercalated with lacustrine clays and other

continental beds. As Reinhold²⁷ pointed out, the diatoms in these various units largely reflect the gradual emergence of Java. Previous work by us^{28,29} has tied Late Pliocene and Pleistocene stratigraphic events (datum levels) in the Tropical Indo-Pacific to the palaeomagnetic reversal and oxygen isotope records^{30,31}. The use of these two standards tied to diatom datum levels permits us to draw correlation lines for the Late Pliocene and Pleistocene of the Tropical Indo-Pacific with a high degree of reliability. Within this context, we studied the marine diatoms in Reinhold's original samples from the Upper Kalibeng Beds and from the marine intercalations in the overlying Putjangan Beds (Fig. 2). These were compared with diatom ranges in a central Pacific core (V28-179) which has an expanded Late Pliocene section and which has been analysed for palaeomagnetism and oxygen isotopes³¹. The lower part of the Upper Kalibeng Beds contains *Rhizosolenia praebertsonii*, *R. praebertsonii* var. *robusta*, *Thalassiosira convexa* and *Th. convexa* var. *aspinosa* suggesting a correlation with the basal part of the Matuyama Reversed Epoch. The upper parts of the Upper Kalibeng Beds, on the other hand, contain only two stratigraphic markers (*R. praebertsonii* and *R. praebertsonii* var. *robusta*), thus placing it after the last appearance of *Th. convexa* and *Th. convexa* var. *aspinosa*. The absence of *Pseudoeunotia doliolus* and of the recurring species *Coscinodiscus nodulifer* var. *cyclopus* suggests that the contact between the Upper Kalibeng Beds and the Putjangan Beds (and, thus, the transition between marine and non-marine sediments) should be dated after the last occurrences of *Th. convexa* and *Th. convexa* var. *aspinosa* and *C. nodulifer* var. *cyclopus* and before the first appearance of *P. doliolus* and reappearance of *C. nodulifer* var. *cyclopus*. Within this context, our data suggest that the base of the Putjangan Beds in Eastern Java falls between approximately 1.9 and 2.1 Myr.

As the earliest hominid fossils occur in lake sediments of the Putjangan Beds which overlay Pliocene marine sediments, early man must have migrated to Eastern Java soon after the local emergence. The age of 1.9–2.1 Myr given by diatom analysis for this emergence must be considered tentative, however, pending more detailed sampling for micropalaeontologic and palaeomagnetic study. Further, since the uplift of Java proceeded from west to east⁸ a micropalaeontological and palaeomagnetic study of the marine/non-marine transition in several sections could provide us with a more detailed history of the emergence of this island during the Late Cainozoic³¹.

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Minor metal content of surface seawater particulates and organic-rich shelf sediments

CERTAIN sediments deposited under areas of high primary productivity contain considerably more organic carbon than those laid down in other marine environments; for example, diatomaceous clays on the continental shelf off South-West Africa (Namibia) have an average of 9% organic carbon¹. Calvert and Price² have reported that these sediments also have enhanced concentrations of some trace metals, such as Cu, Zn and Pb. During the past few years we have been carrying out collections of surface (0–5 m) seawater particulates from various areas of the World oceans^{3–6}. Here, we present average data on the concentrations of Al, Cu, Zn, Pb and organic carbon in a series of particulates collected off the coast of West Africa, and compare them to organic-rich marine sediments.

Calvert and Price compared the modern environment of organic-rich sedimentation on the continental shelf of Namibia to that prevalent during the deposition of some ancient bituminous shales. According to Brongersma-Saunders^{7,8} the enrichment of Cu, Pb and Zn in many of these deposits, such as the Permian Kupferschiefer of northern Germany, is the result of their supply by plankton in regions of upwelling. However, Calvert and Price² found that the concentrations of Cu, Zn and Pb in the sediments on the South-West African shelf were not enriched to the same extent as in the Kupferschiefer, and concluded that the same mechanism of trace element deposition had not been operative in the two areas. Nonetheless, Calvert and Price² found a high degree of correlation between the Cu, Pb and Zn and the organic carbon concentrations in their sediments, and suggested that the metals were intimately associated with the organic fractions of the deposits. They stressed, however, that before the mechanism of deposition could be identified, more data were required on the relative contributions made to the total metal concentrations by biogenous, terrigenous and authigenic sources. In this respect, the concentrations of Cu, Zn and Pb in the suspended material of the overlying waters is of critical importance. Data have been

provided on the trace metal contents of plankton from various regions of the World oceans⁹, and although the metal concentrations vary considerably from one region to another certain overall enrichment trends are apparent. However, there is a general lack of information on the composition of total particulate material, a mixture of lithogenous, hydrogenous and biogenous components, from regions of high primary production.

We made particulate collections by a continuous centrifuge technique described elsewhere⁶; Cu, Zn, Pb and Al were determined by either atomic absorption or spectrographic methods, and organic carbon was measured using a C–H–N analyser. The samples selected for the sediment comparison were all collected on transects within ~500 km of the coast of West Africa between 8 and 30° N, during April, July and October 1972 and February 1973.

None of the particulates were collected off the coast of South-West Africa. However, the sample transects were chosen such that they are all within the general region of high primary production lying off the coast of West Africa (5–30° N)¹⁰. The particulates should, therefore, offer a reasonable comparison with the organic-rich South-West African shelf sediments if theories of planktonic sedimentation are to have a general ocean-wide application to the genesis of organic-rich near-shore sediments and their lithified equivalents. In other words, the two data sets, that is, those of the particulates and the shelf sediments, can be used to test the geographical independence of the general mechanisms which result in the correlation between organic matter and certain trace metals in sediments underlying areas of upwelling.

Table 2 The maximum minor element/organic carbon ratios in South-West African shelf sediments and the average ratios in northeastern Atlantic surface water particulates

Element/organic carbon (% × 10 ³)	Northeastern Atlantic particulates	Diatomaceous clays, South-West African shelf*
Cu	0.51	0.56
Zn	1.0	1.8
Pb	0.23	0.29

*Data from Calvert and Price².

The average concentrations of Cu, Zn, Pb, Al and organic carbon in the South-West Africa shelf sediments and the northeastern Atlantic particulates are given in Table 1. This shows that the sediments are not composed simply of settled undifferentiated bulk samples of the surface water particulates. For example, the sediments have a much larger 'terrestrial' component, represented by Al, and a much smaller 'planktonic' component, represented by organic carbon, than do the particulates. This is not unexpected because of the various diagenetic processes which affect the deposited sediments. However, in addition to bulk samples, Calvert and Price² have given data on the maximum concentrations of Zn, Cu and Pb in the sediments, together with that of organic carbon. If these maximum values are assumed to offer the best available estimate of the extreme situation in which the plankton have retained their original composition, they can be directly compared to the surface water particulates. Such a comparison is made in Table 2, from which it can be seen that the metal/organic carbon ratios in the particulates are similar to those in the maximum metal-enriched sediments.

The initial composition of plankton-rich surface water particulates is usually considerably modified, after deposition, by diagenetic processes. However, the data presented here strongly suggest that modern organic-rich sediments underlying regions of high primary production can receive a substantial proportion of their Cu, Zn and Pb from the sinking of relatively undifferentiated surface water particulates of which organic components,

Table 1 The partial element average compositions (p.p.m.) of South-West African shelf sediments and northeastern Atlantic surface water particulates

	12 Northeastern Atlantic particulates	Diatomaceous clay South-West African shelf*
Al	9,700	15,446
Cu	112	68
Zn	225	68
Pb	50	12
Organic C	219,000	93,500

*Data from Calvert¹.

including plankton and faecal pellets, are an important constituent.

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Diversity in constant and fluctuating environments

It has been suggested that environmental fluctuations will limit the degree to which species may be packed; the closeness of the packing increasing with increasing environmental stability¹. This theory has been challenged by the suggestion that although there is a limit to the degree of niche overlap in the real world, the limit is only sensitive to severe environmental fluctuations². The fluctuations are regarded as severe when their variance to mean ratio is greater than or equal to 0.3 (ref. 2). We report here our studies on populations of ciliate protozoa of a freshwater community maintained in temperature-controlled microcosms. Comparisons were made between the changes in diversity of the ciliates in a microcosm maintained in a fluctuating temperature and the changes taking place in a control maintained at a constant temperature. The results suggest that the diversity of the ciliates is significantly greater in the fluctuating-temperature system than in the constant-temperature system.

Samples of water were taken from Sand Loch, Aberdeenshire (grid ref. NJ 034284), and brought back to the laboratory. The water was filtered to remove large pieces of debris before being put into the experimental system. (The mesh of the filter was square, with sides of 250 μm .) Two sets of samples were collected, the first in January 1978 (experiment 1) and the second in April 1978 (experiment 2).

The water samples were maintained in batch culture in four 20-l flasks, which were kept in one of two water baths, the temperature of each being independently thermostatically controlled. The fluctuating environment was created in one bath by daily variations in the temperature (Figs 1 and 2) ranging over 2–12 °C, with a complete cycle of variation occurring every 2 d. The control system was maintained at 7 °C. In each system, samples were taken from one flask while the other acted as a reservoir to maintain a constant culture volume in the sampled vessel. A sample of 0.25 l was taken from each system daily and sedimented overnight according to the method described by Lund, Kipling and LeCren³. The ciliates present in each sample were identified (to genus level) and counted during the day after

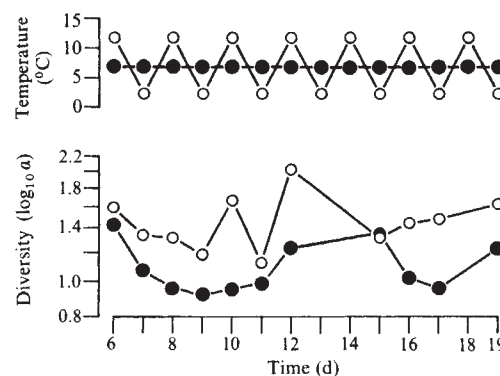


Fig. 1 Changes in temperature and diversity with time during expt 1. The lower graph shows the change in diversity ($\log_{10} \alpha$) with time (d) for the fluctuating-temperature system (○) and for the constant-temperature system (●). The upper graph shows the change in temperature (°C) with time (d) for the two temperature regimes, fluctuating temperature (○) and constant temperature (●).

sampling. The diversity of the ciliates present in the cultures was estimated using the log-series parameter, α (Figs 1 and 2). This index was used in preference to the Shannon–Wiener information index⁴ because the former does not overemphasise the presence of the commonest taxa to the same extent as the latter^{5,6}. Also, it has been shown that the site-discriminating ability of α is superior to the information index⁵ and is, therefore, more applicable in this type of investigation.

Determination of the between-system to within-system variance (Table 1) showed that variation between the diversity of the ciliates in the two temperature regimes was significantly greater than that within each of the two systems in both experiments ($P < 0.01$ in experiment 1, $P < 0.05$ in experiment 2). The diversity of the two ciliate populations did not differ significantly initially, but it was found that over a period, the diversity of the ciliate populations in the fluctuating-temperature system was significantly greater than that of those maintained in a constant temperature (in both experiments $P < 0.01$). A Spearman rank correlation showed that in the first experiment there was no correlation between the diversity of the ciliate populations in either temperature system and time, but that in the second experiment, there was a significant positive correlation between diversity and time for both ciliate populations ($P < 0.01$). These differences may be accounted for by the differing composition of the ciliate populations due to seasonal variation in the freshwater community.

The results obtained from both these experiments suggest that, in the conditions of these experiments at least, deterministic perturbation of an environmental factor leads to a

Fig. 2 Changes in temperature and diversity with time during expt 2. The lower graph shows the change in diversity ($\log_{10} \alpha$) with time (d) for the fluctuating-temperature system (○) and for the constant-temperature system (●). The upper graph shows the change in temperature (°C) with time (d) for the two temperature regimes, fluctuating temperature (○) and constant temperature (●).

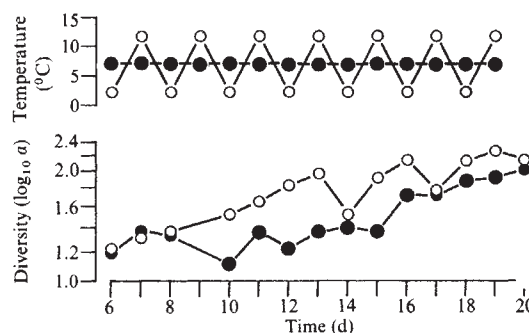


Table 1 Analysis of variance for experiment 1 giving the between-environment to within-environment variance ratio (F) for experiments 1 and 2

Source of variation	d.f.	Sums of squares	Variance s^2	F
Between environments	1	0.77	0.77	
Within environments	20	0.93	0.05	
Total	21	1.70		
Expt 1				15.4*
Expt 2				4.8†

* $P < 0.01$.† $P < 0.05$.

greater diversity of the ciliate population. The decreased index of diversity in the constant-temperature system is due to the increasing relative abundance of a small number of genera which dominate the ciliate populations. In the fluctuating-temperature system the relative abundances of the genera present are comparatively lower than those in the control system, and domination of the ciliate populations by a small number of genera does not occur to the same degree. It would seem that in the fluctuating-temperature system the environmental variations may be reducing the number of individuals present such that competition plays only a minor part in population control, thus leading to more even abundances of the genera present. In contrast to this, the constant-temperature system has allowed the part played by competition in population control to increase, such that a few genera have competitively excluded the others and so have become dominant in the ciliate population.

This reasoning is supported by Connell's assertion⁷ that the high species diversity of tropical rain forests and coral reefs is caused by infrequent catastrophic environmental perturbations which keep the community in a nonequilibrium-nonclimatic state, thus reducing the chance of elimination of less-able competitors, as is suggested by these experiments which use deterministic and less extreme perturbations.

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Attraction of the tick *Ixodes neitzi* to twigs marked by the klipspringer antelope

THE klipspringer (*Oreotragus oreotragus*), a small African antelope which lives in specific rocky outcrops, secretes a dark pungent resin-like substance from the ante-orbital glands which is utilised as a means of intraspecific communication¹. Marking is carried out by rubbing the ante-orbital glands on a substrate such as the twigs of shrubs on which the secretion is deposited. We have now observed that the adults of the tick *Ixodes* (*Afrimedes*) *neitzi*² aggregate on twigs which have been previously marked by the klipspringer (Fig. 1). The attractive component of

the secretion is water soluble. The capability of the adult tick to detect the klipspringer scent was demonstrated in field observations and with laboratory experiments.

Adults of *I. neitzi* were collected from shrubs in the Rhodes Matopos National Park, Rhodesia. *Securinea virosa* was the dominant plant species in this area and grew from cracks in the rocks or closely to rocks.

The effect of the resin-like secretions collected from the twigs was assessed using the petri dish technique³ with minor modifications. Each 9-cm Petri dish was marked into eight sectors and because *I. neitzi* were very sensitive to desiccation, moistened discs of filter paper were placed in each sector. At the start of each experiment, 10 ticks (male or female) were placed in the centre of the petri dish. The tested materials were always placed in sector No. 8. A concentrated aquatic extraction of twigs marked by the klipspringers was the only substance which significantly attracted the ticks (Fig. 2).

Further examination using a Y-shaped olfactometer^{4,5} showed that none of the substances tested were attractive ($P < 0.5$, t -test for paired observations). The failure of the ticks to detect extract in the olfactometer and the fact that *I. neitzi* adults did not aggregate around freshly applied deposits which had been moistened, suggest that the scent secretion of the klipspringer is not airborne and only becomes attractive to the ticks or any other arthropods after a copious amount of water had been applied to the extract.

The solid secretion was extracted with different solvents and only the water soluble fraction was active in attracting the ticks (Fig. 3).



Fig. 1 a, Illustration of a twig marked by scent secretion from the ante-orbital gland of klipspringers. b, Assembly of *Ixodes neitzi* adults to a marked twig.

Uncontaminated branched twigs from the same shrubs from the experimental area were selected. The base and left arm of the Y-shaped twigs were painted with a concentrated aqueous solution prepared from marked twigs collected in the field. The Y-shaped twigs were positioned in soil with the two arms pointing upwards. Adult ticks were released on the soil and allowed to migrate. As the ticks moved up the vertical structure they were able to select one of the arms of the twigs.

These experiments were conducted in the field and repeated in the laboratory. In the field $66.2 \pm 2.9\%$ ($P < 0.001$) of the adults released in the vicinity of the Y-shaped twigs ascended the arm on which the aqueous klipspringer scent extract was applied. In the laboratory similar results were obtained where $63.7 \pm 1.00\%$ ($P < 0.001$) of the adults selected the treated arm. With similar but untreated Y-shaped twigs $52.2 \pm 2.08\%$ ($P < 0.3$) of the adult ticks selected the left arm.

In some *Ixodes* species an assembly pheromone has been shown to be secreted before feeding commences^{6,7}. To exclude any possibility of prior contamination of the marked twigs with a tick assembly pheromone, an aqueous extract was made from the ante-orbital glands of a klipspringer. When this material and

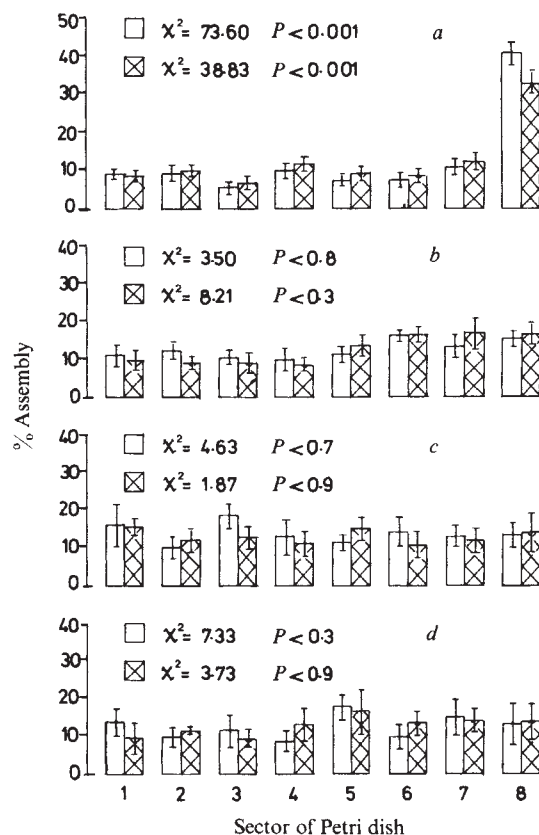


Fig. 2 Average assembly response (% $\pm$ s.e.) of *Ixodes neitzi* females (□) and males (⊠) to various materials placed in sector 8. *a*, Concentrated aquatic extract (30%) of scent marks of klipspringer. *b*, Dilute aquatic extract (10%) of scent marks of klipspringer. *c*, Scent marks of klipspringer which had been dipped in water before the experiment. *d*, Solution of unmarked twigs (control).

an aqueous extract of marked twigs were compared no differences were found, showing that the attractive principle arose from the klipspringer orbital gland secretions and excluding the possibility of pheromone contamination from the tick.

The klipspringer exudate apparently solidifies rapidly after being deposited on the tips of the twigs (Fig. 1a). This substance is subjected to the heavy rains falling between November and March (800 mm). The active component dissolves in the water, drips or runs down through the branches and stems to the

ground level. We assume that the adult ticks, probably by using a gustatory sense, respond to the extract and follow it to the tip of the twigs. The adults remain inactive on the twigs until the return of the klipspringer to re-mark the area. Sniffing of the marked twigs by the klipspringer and consequent physical movement of the shrubs, stimulate the tick to gain access to the host.

This constitutes the first report of a tick species locating its mammalian host by detecting a specific chemical compound or compounds, used by the host as a communicative marking signal, thus increasing its probability of survival in that particular habitat.

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Mixed ligand chelate therapy for plutonium and cadmium poisoning

SERIOUS environmental and industrial hazards are associated with the release of radioactive^{1–3} and nonradioactive^{4–7} metals. Most treatments for acute and chronic metal poisoning use chelating agents forming soluble, stable and excretable metal complexes^{1–9}. Although a few chelants are used clinically⁸, the efficacy of single-chelant therapy and variations is limited^{1–3} and deleterious effects can occur^{4–7,9,10}. Consequently, Schubert recommended^{11,12} the use of mixed-ligand chelates (MLCs), in which the metal binds two or more different chelants, because of the enhanced stabilities attainable. For example, formation constants of thorium MLCs can exceed that of EDTA alone by at least 10^{13} (refs 13–15). We report here hitherto unparalleled achievements using MLC treatment, namely, complete removal of tissue deposits of ²³⁹Pu and prevention of mortality in animals given lethal, $>LD_{100}$, doses of cadmium.

Female, HA(ICR) mice, 45 d old, weighing 25.1 ± 0.6 g, were injected intravenously with plutonium or cadmium solutions; chelants were usually administered intraperitoneally. Monomeric^{16–18} ²³⁹Pu(IV) in 1% sodium citrate and cadmium chloride with and without addition of ¹⁰⁹Cd were used. Radiochemical assays¹⁹ were made from wet ashed tissues and excreta, and the activity measured in a liquid scintillation counter.

Fig. 3 Average assembly response (% $\pm$ s.e.) of *Ixodes neitzi* females (□) and males (⊠) to various materials placed in sector 8. *a*, Water soluble fraction of the scent marks of the klipspringer. *b*, Methanol soluble fraction of the scent marks of klipspringer.

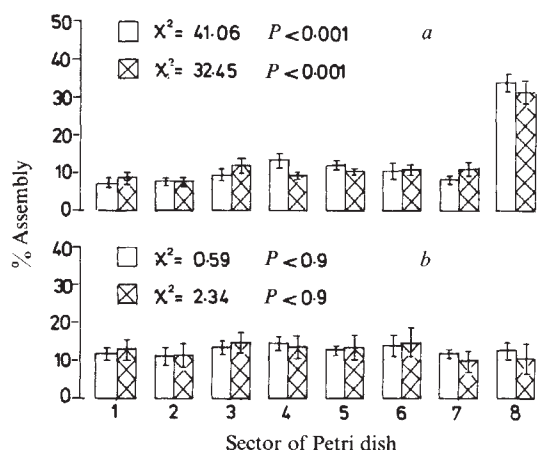


Table 1 Effect of single and mixed ligand chelate treatment on the tissue levels and total excretion of ^{239}Pu in female mice after Pu administration

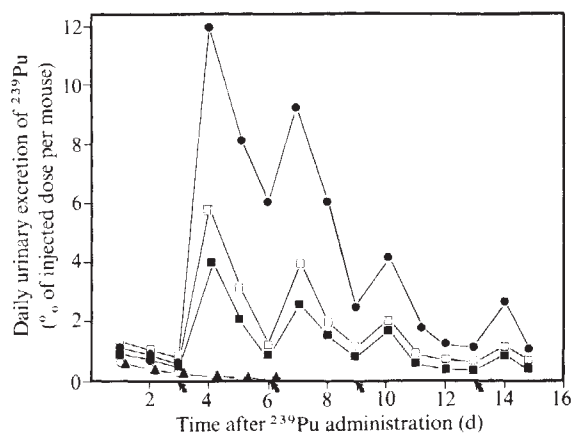
Sample	% Of injected dose of ^{239}Pu				
	Untreated controls	SA(2.0)	DTPA (0.5)	DTPA (2.5)	DTPA + SA (0.5 + 2.0)
Urine	4.3	5.4	24.8	28.4	54.0
Faeces	28.2	26.2	41.4	44.6	46.6
Liver	5.4 ± 0.3	5.1 ± 0.5	0.33 ± 0.1	0.22 ± 0.1	0 ± 0.01
Skeleton	33.7 ± 1.3	33.5 ± 2.1	13.8 ± 0.5	13.6 ± 0.4	0 ± 0.1
Total	71.6	70.2	80.3	86.8	100.6
Body (calculated)	67.5	68.4	33.8	27.0	0

The chelate treatment shown is in mmol per kg, and was given twice weekly. Measurements were taken 33 d after Pu ($0.0162 \mu\text{Ci}$ per mouse) administration and 30 d after the onset of treatment. The liver and bone levels represent average values $\pm$ s.d. from six mice, and the urine and faeces represent values per mouse from analyses on pooled samples from three mice. The total skeletal content was obtained by multiplying the amount of Pu in both femurs by 12 (refs 17, 18). The body values were calculated by subtracting the urine + faecal values from 100. See text and legends to Figs 1 and 2 for experimental details.

Polyaminopolycarboxylic acids (PAPCs) were administered as calcium-sodium salts to prevent hypocalcaemia, and components of MLCs were pre-mixed, that is, injected as a single solution.

Three days after Pu administration, when almost all had been deposited in the tissues^{16-18,20}, twice weekly injections of MLC [diethylenetriaminepentaacetic acid (DTPA) and salicylic acid (SA)] and of the individual chelants were initiated. Daily urinary excretion (Fig. 1) demonstrated that DTPA + SA increased Pu excretion to levels far above those induced by DTPA alone; SA alone had no effect (Fig. 1). By day 33 post-Pu, all injected Pu had been eliminated in urine and faeces (Table 1). After 10 treatments with massive doses of DTPA, skeletal Pu remained at 15% of the injected dose, whereas that of DTPA + SA-treated mice was, within counting statistics, free of Pu (Fig. 2 and Table 1). The livers of MLC-treated mice were devoid of Pu after four treatments. When treatment was delayed until 6 d post-Pu, 12 DTPA treatments reduced skeletal Pu to $24.3 \pm 0.3\%$, in agreement with previous work²¹, compared to $1.4 \pm 0.2\%$ of injected dose after 12 MLC treatments.

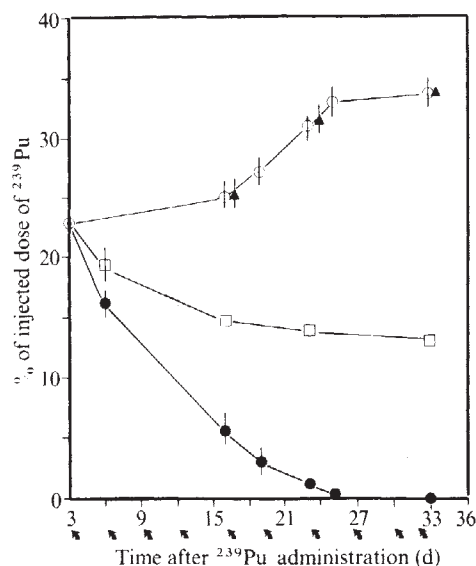
Fig. 1 Effect of single and mixed ligand chelates on the daily urinary excretion in female mice of plutonium-239 (specific activity $0.063 \mu\text{Ci} \mu\text{g}^{-1}$). The background count was 23.0 ± 0.5 d.p.m. Each point represents ^{239}Pu values per mouse from pooled samples of three mice. The animals were given a single intravenous injection of monomeric Pu(IV)-citrate of $0.0162 \mu\text{Ci} = 35,600$ d.p.m. The counting efficiency was almost 100%, as determined with calibrated standards. Three days later, treatment by intraperitoneal injection began and continued twice weekly. The DTPA was administered as the CaNa_3DTPA salt and that of SA as the sodium salt. The DTPA + SA were pre-mixed and injected as a single solution. ●, DTPA + SA (0.5 mmol per kg + 2.0 mmol per kg); □, DTPA (2.5 mmol per kg); ■, DTPA (0.5 mmol per kg); ▲, SA (2.0 mmol per kg); ○, Pu controls; arrows indicate treatment.



In experimental, acute Cd poisoning, DTPA or EDTA are marginally effective^{2,3,22-25}, and dimercaptopropanol is contraindicated^{4,8,25}. MLC treatment of mice 1 h post-Cd with EDTA + SA or EDTA + 2,3-dimercaptopropanol-1-sulphonate (Dimaval) (DMPS) resulted in 100% survival, whereas all single-chelant-treated animals died (Fig. 3). The therapeutic effect of the MLCs reflected a strong decorporative action. Thus, after a dose of ^{109}Cd -labelled chloride which was 4.5 times the LD_{100} , a single EDTA + SA injection 1 h later removed 82% of the Cd present in the kidneys at the onset of treatment; EDTA was ineffective.

The effectiveness of MLC therapy is maintained when low, clinically accepted doses¹⁻³ are used; for example, 0.05 mmol per kg for PAPCs and 0.2 mmol per kg for secondary chelants. The MLCs are also effective when administered orally. Many

Fig. 2 Effect of single and mixed ligand chelate treatment on the concentration of ^{239}Pu in the skeleton of female mice. Each point represents the average value of analyses made on the femurs of each of six mice. The skeletal values were obtained by multiplying the femur values by 12 (refs 17, 18). As the skeletal levels of ^{239}Pu for DTPA doses of either 0.5 mmol per kg or 2.5 mmol per kg were almost identical, one symbol was used for both. The SA dose was 2.0 mmol per kg, and the MLC was 0.5 mmol per kg DTPA + 2.0 mmol per kg SA. The error bars represent the standard deviation and are not included when the symbol diameter exceeds the magnitude of the standard deviation. Treatment with single or mixed ligand chelants began 3 d after the intravenous injection of $0.0162 \mu\text{Ci}$ of ^{239}Pu in the monomeric citrate form. Further experimental details are given in the text and legend to Fig. 1. ○, Pu controls; ▲, SA; □, DTPA; ●, DTPA + SA; arrows indicate treatment.



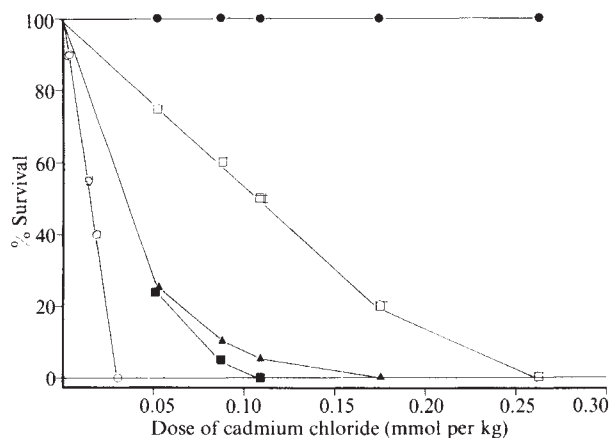


Fig. 3 Effect of treatment with single and mixed ligand chelates on the survival of female mice given increasing doses of cadmium. Each point represents the % survival of a group of at least 20 animals. The mice were given a single injection in the tail vein, of an aqueous solution of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (MW 228.3). One hour later each mouse received a single, intraperitoneal injection of the specified chelant or mixed chelant solution. The untreated controls received a single i.p. injection of isotonic saline solution. Animals which survived 3 d remained healthy for the remainder of the observation period (4 weeks). ●, EDTA + SA or EDTA + DMPS; □, EDTA; ▲, SA; ■, DMPS; ○, Cd controls.

MLCs consisting of PAPCs with bidentate aromatic compounds or other bidentate compounds or PAPCs are at least as effective for Pu decorporation and therapy of other metals including Cu, Zn, La, Fe(III), Ni, Pb and Th, (these MLC systems include EDTA or DTPA combined with 1,2-diamino-cyclohexanetetraacetic acid, dithiocarb, hydroxyethylene-diaminetriacetic acid, nitrilotriacetic acid, penicillamine, salicylhydroxamic acid, sulphosalicylic acid and Tiron.) We are also investigating combinations of MLCs for decorporation of polymeric and insoluble forms of plutonium and other metals largely resistant to single-chelant treatment¹⁶⁻¹⁸ by using Fe-effective MLCs to facilitate decorporation of metals, for example, Pu, associated with endogenous iron species such as transferrin and ferritin.

Many chemical considerations play a part in the choice of MLC systems. For example, for 6-coordinate Cd(II), the MLC of EDTA + SA (total dentateness 8), as anticipated²⁶⁻²⁹, is more effective than DTPA + SA (total dentateness 10), as the presence of four unbound carboxyl groups in DTPA + SA, compared to two in EDTA + SA, is de-stabilising. The greater stability of many MLCs, larger than that predicted from statistical factors^{30,31}, derives^{13-15,26-31} from charge neutralisation, steric effects, intramolecular electrostatic interactions and cooperative electronic effects, particularly with aromatic secondary chelants. As the secondary chelant alone was largely inactive, we conclude that the primary metal chelate facilitates incorporation of the secondary chelant, presumably by deprotonation.

Experimental evidence of *in vitro* and *in vivo* formation of MLCs is provided by different chemical and biological approaches. When the total dentateness of the chelants was less or greater than the liganity of the metal ion, for example, the metal chelates were hydrophilic, but they were lipophilic when the total dentateness was equal. Experimentally, we found that Pu(EDTA), Pu(SA) and the MLC of Pu(DTPA)SA were indeed hydrophilic, and that Pu(DTPA) and Pu(EDTA)SA were lipophilic as determined from partition coefficients using H_2O as the polar phase and *n*-heptane as the nonpolar phase. Ultrafiltration experiments^{11,12,32} on liver homogenates containing Pu or Th demonstrated greater effectiveness of MLCs relative to single chelants, and the superiority of EDTA + catechol (CAT) or DTPA + CAT relative to EDTA + SA or DTPA + SA, in agreement with potentiometric titration studies¹³.

In vivo MLC formation was demonstrated with yeast-fermented rats³³ in which varying concentrations of injected Zn(EDTA) or Cu(EDTA) reduced salicylate antipyresis to a predictable, quantitative degree³⁴. Removal of endogenous metals such as Zn by MLCs is similar to that by EDTA and DTPA (refs 2, 35-37), but treatment twice weekly, because of dietary replacement, produced no toxic symptoms. Toxicological and pharmacokinetic studies on MLCs using ^{14}C - and ^3H -labelled compounds are underway.

Mixed ligand chelate or complex formation by naturally occurring ligands and metals enters into biochemical processes and environmental situations, such as transport of metals from soils into the food chain and ground water. The ability of MLCs to remove all ^{239}Pu permits investigation of the mechanisms of tumour formation by Pu (refs 2, 3, 38) and modification of the genetic-toxicological effects of metals generally. The large variety of MLC systems available opens the way for major advances in the prophylaxis and treatment of metal toxicity, radioactive imaging with γ -emitting radiometals and diagnosis of exogenous metal body burdens by provocative or challenging doses⁴.

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Evidence that the TSH receptor acts as a mitogenic antigen in Graves' disease

GRAVES' DISEASE (GD) is an autoimmune disorder associated with the formation of thyroid antibodies, one class of which, thyroid-stimulating antibodies (TSAb), is thought to be involved in the pathogenesis¹⁻³. TSAb act similarly to thyrotropin (TSH) on the thyroid follicle cell through the mediation of adenylate cyclase⁴. The formation of TSAb is thought to be due to a change in the immune tolerance or to some alteration of the antigen, which has been suggested to be the TSH receptor². Some experimental evidence favours a defect in the immune surveillance, caused by decreased suppressor T-cell function^{2,5}. It has been reported that sensitised lymphocytes from patients with GD can produce TSAb *in vitro* after nonspecific stimulation^{6,7} as well as after specific stimulation with thyroid homogenate⁸. We report here that the lymphocyte-stimulating effect of thyroid membranes is abolished if the thyroid membranes are preincubated with TSH. According to our hypothesis, this means that the TSH receptor is the mitogenic antigen.

Eleven patients with GD and seven with toxic nodular goitre (TNG) were studied. The patients were hyperthyroid according to clinical and laboratory data (Table 1). The grouping of the patients into GD and TNG was based on clinical signs and on thyroid palpation and scintigraphy. Lymphocytes from these patients were studied with the lymphocyte transformation test. The method is described in the legend to Fig. 1. The stimulation index (SI) was calculated by dividing the mean c.p.m. of the three test cultures by the mean of the three control cultures. The mean lymphocyte SI for 10 healthy persons tested with thyroid membranes as the antigen was 1.02 ± 0.22 (mean $\pm$ s.d., range 0.61–1.35). The stimulation was considered positive when SI was more than 1.50, that is, mean + 2 s.d. In control incubations with leukoagglutinin (LA) as the mitogen, the SI varied from 20 to 90, reaching its maximum on day 3, and from 3 to 10 on day 7. SI with purified protein derivative of tuberculin (PPD) varied

Table 2 The effect of preincubation of LA and PPD with TSH

LA	Stimulation index with		
	LA + TSH	PPD	PPD + TSH
52.9	62.9	4.5	4.2
38.0	36.4	2.3	1.8
34.8	32.1	1.7	1.7
26.2	25.3	15.0	13.0
26.1	24.9		
32.0	36.8		

For further details of the lymphocyte transformation test and preincubations see the legend to Fig. 1.

from 1.7 to 15. The membranes from kidneys did not show any lymphocyte-stimulating effect in any patients tested. The coefficient of variation between triplicates was 4.1%.

When thyroid membranes were used as antigen, the mean SI was 3.64 in the patients with GD, nine of whom showed a positive response. A positive response was also seen in two patients with TNG (Table 1). Figure 1 shows the results for the 11 patients with a positive lymphocyte transformation test when leukocytes were incubated with thyroid membranes and with membranes which were preincubated with TSH. The lymphocyte stimulation disappeared completely in eight cases and partly in three. Thus, it seems that preincubation of the mitogenic membrane preparation with TSH blocked the mitogenic determinant. Preincubation of the thyroid membranes with human chorionic gonadotropin (hCG), which has the same α -subunit as TSH, failed to influence the stimulating effect of membranes (Table 1). Preincubation of LA or PPD with TSH did not substantially interfere with the mitogenic activity of either LA or PPD (Table 2).

The fact that preincubation with TSH inhibited the stimulating effect of the thyroid membranes on lymphocytes from patients with GD is considered strong evidence in favour of the hypothesis that autoimmunisation against the TSH receptor on the thyroid follicle cell membrane is the pathogenic mechanism of hyperthyroidism in Graves' disease. In other words, it seems

Table 1 Diagnosis and stimulation index in patients with Graves' disease or toxic nodular goitre

Patient	Sex	Age	Diagnosis	PBI (0.32–0.63 mol l ⁻¹)	T ₄ (50–135 nmol l ⁻¹)	FT ₄ (30–85 pmol l ⁻¹)	Lymphocyte transformation test		
							Stimulation index with	Membranes preincubated with TSH	Membranes preincubated with hCG
							Thyroid membranes		
L.B.	F	46	GD		253		2.16	1.40	2.20
A.L.	F	33	GD		250	450	1.77	1.00	1.70
H.T.	F	38	GD		246		0.40	0.47	
O.R.	M	34	GD		205		2.10	1.00	
L.R.	M	52	GD	1.06			3.00	1.86	
J.T.	F	36	GD		148	208	2.00	0.40	
F.B.	F	29	GD	1.25			1.00	0.40	
K.V.	M	66	GD		177		5.40	0.90	
S.P.	M	62	GD	0.72			11.10	0.60	
R.B.	F	41	GD		151		4.50	2.40	
K.A.	F	19	GD		280	158	6.55	1.60	
E.L.	F	34	TNG	0.54			1.70	0.90	1.70
V.T.	M	52	TNG		232		14.70	1.10	
F.A.	F	72	TNG		152	154	0.20	0.10	
J.P.	F	27	TNG		183	199	1.00	0.40	
B.J.	F	68	TNG		131	135	1.00	0.30	
P.M.	F	72	TNG	0.86			0.66	0.30	
E.V.	F	31	TNG	0.95			1.00	0.30	

The diagnosis of hyperthyroidism was based on clinical symptoms and signs as analysed by Lamberg *et al.*¹⁴. The diagnosis was confirmed by at least one of the following tests: serum protein-bound iodine (PBI), total thyroxine (T₄), and free thyroxine index (FT₄) as calculated from T₄ and triiodothyronine uptake by Sephadex (T₃U)¹⁵. The normal values are given in parentheses under the headings. The division into toxic Graves' disease (GD) and toxic nodular goitre (TNG) was made by palpation and thyroid scintigraphy. The lymphocyte transformation test was carried out within 2 months of diagnosis. For further details of the lymphocyte transformation test see legend to Fig. 1.

that lymphocytes of patients with GD are sensitised against the patients' own TSH receptor molecule, thus inducing TSAb production. TSAb obviously cause hyperthyroidism in most patients with GD. Some patients, however, do not develop hyperthyroidism, possibly because the functional capacity of the thyroid is impaired by coexisting destructive autoimmune processes^{9,10}. Another possible reason for the lack of response is that in some patients the TSAb are merely blocking antibodies¹¹, which do not stimulate the thyroid adenylate cyclase system.

The fact that the lymphocyte transformation test was positive in only two cases with TNG indicates that this type of hyperthyroidism is not usually due to a breakdown of the immune tolerance against the TSH receptor. However, nodular goitre

and GD may coexist, as shown in studies on a long-acting thyroid stimulator¹². In the present study, one of the two cases with TNG who had a positive SI was also tested by radioreceptor method for TSAb¹³: a positive TSAb index was found.

We do not know why the self-tolerance against TSH receptors is broken, nor why GD is self-limiting. The pathogenesis of TNG is unlikely to be related to the autoimmunisation phenomena seen in GD, but nodules may exist in a GD gland.

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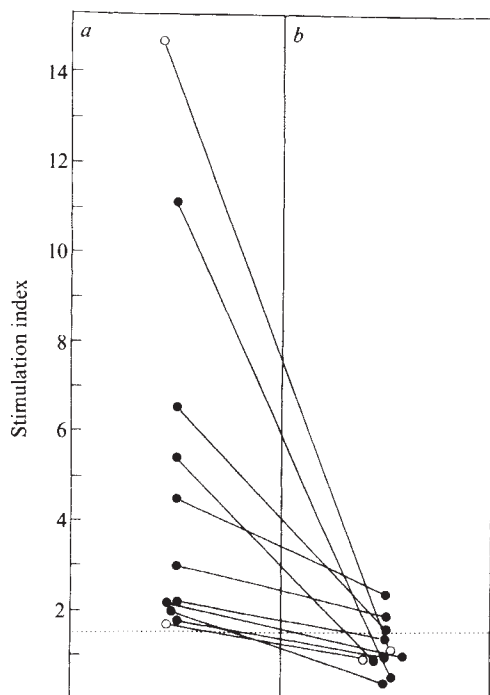


Fig. 1 The effect of preincubation of thyroid membranes with TSH on their stimulating ability on lymphocytes from nine patients with Graves' disease (●) and two patients with toxic nodular goitre (○). *a*, Stimulation index (SI) after incubation with thyroid membranes; *b*, SI of lymphocytes from the same patients after preincubation with TSH. 40 ml of blood was defibrinated with glass beads and mixed with 6 ml of a 6% dextran (MW 110,000) solution and allowed to sediment for 1 h at 37 °C. The leukocyte-rich plasma was collected and the leukocytes were washed three times in 5 ml Hanks' balanced salt solution and suspended in Eagle's minimal essential medium (MEM) containing 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, 2 mM L-glutamine and 10% fetal calf serum (Flow). Triplicate cultures of 1 ml containing 2 × 10⁶ leukocytes were incubated for 7 d with or without added antigen. After 6 d incubation at 37 °C in a humidified atmosphere (5% CO₂, 95% air) 1 µCi of ¹²⁵I-labelled deoxyuridine was added to each tube. 18 h later the cells were collected on to glassfibre filter papers using a multiple automated sample collector. The filters were dried at 60 °C and counted in a gamma counter. The antigen used was a thyroid membrane preparation (10,000g)¹³ which was formalinised according to the method of Ross *et al.*¹⁶. Control experiments showed that formalinisation of the membranes did not influence their ability to bind TSH specifically. The antigen was used at a final concentration of 50 µg protein per ml of medium. The thyroid antigen was also preincubated with 500 mU TSH (Actyon, Ferring) to block the TSH receptors on membranes. Incubation was carried out for 20 min at 20 °C and used at the same concentration as the thyroid membranes alone. The control experiments with LA (Pharmacia) and PPD (State Serum Institute, Copenhagen) and the preincubations with TSH were carried out according to the same culture method. The LA concentration used was 5 µg ml⁻¹, PPD 250 IU ml⁻¹, hCG 50 IU and TSH 500 mU.

Graft-versus-host disease impairs cerebellar growth

THE mechanisms of mental retardation are of critical interest to both basic developmental neurobiologists and those in the clinical community concerned with perinatal development. The possibility of a deleterious immunological interaction between mother and fetus has long been recognised as a potential developmental hazard¹. However, there have been no reports of specific conditions which involve immunological injury to the developing brain. Graft-versus-host disease (GVHD) is a model disease that can be induced naturally or experimentally by grafting mature, immunocompetent lymphoid cells into an immature, immunoincompetent host. The failure of the host, for example a newborn rat pup, to recognise the graft as non-self leads to an attack by the grafted cells on host lymphomyeloid tissues, resulting in many symptoms, including wasting, anaemia, alterations of lymphoid organ weights, hyperexcitability and an ataxic-like gait². The last two features suggest possible brain dysfunctions and have motivated our investigation of immunologically induced perinatal brain injury. The rat cerebellum was used here as a model system for the study of brain alterations during GVHD because of its well known cytoarchitecture³. In addition, its postnatal development renders it sensitive to many forms of perinatal stress^{4,5}. In this

Table 1 Effect of graft-versus-host disease on several cerebellar growth parameters

Day killed	Injected cells	Cerebellar weight (mg)	Cerebellar contents (mg)			
			DNA	RNA	Protein	DNAF/ASF
GVHD-producing LNC injected						
9	None	63.3±1.7	0.45±0.02	0.37±0.01	4.4±0.1	0.43±0.03
	FLNC	61.1±1.2	0.48±0.01	0.38±0.01	4.4±0.1	0.46±0.04
11	None	85.4±1.2	0.65±0.02	0.44±0.01	6.7±0.3	0.81±0.04
	FLNC	75.3±1.4*	0.54±0.02*	0.39±0.01*	6.1±0.3*	0.53±0.02*
13	None	110.9±1.7	0.89±0.03	0.54±0.01	7.2±0.1	0.54±0.02
	FLNC	102.6±1.8*	0.77±0.01*	0.47±0.02*	6.4±0.2*	0.35±0.03*
14	None	119.0±2.1	0.95±0.03	0.55±0.02	9.0±0.2	0.76±0.05
	FLNC	82.2±4.2*	0.73±0.07*	0.43±0.04*	7.2±0.4*	0.34±0.03*
Non-GVHD-producing LNC injected						
14	None	121.4±4.8	1.04±0.06	0.60±0.02	8.6±0.6	0.62±0.03
	F ₁ hybrid					
	LNC	124.4±4.4	1.05±0.04	0.58±0.02	8.6±0.4	0.58±0.04

Animals injected with either 40×10^6 Fischer lymph node cells (FLNC) or F₁ hybrid LNC were compared with non-injected littermates. DNAF/ASF is the ratio of c.p.m. per g in the DNA fraction to c.p.m. per g in the acid-soluble fraction 1 h after an i.p. injection of ^3H -TdR (2 μCi per g body weight). Results are expressed as mean $\pm$ s.e.m.

* Significantly less than non-injected controls (None), $P < 0.05$.

study, we demonstrate that significant alterations in the cerebellar cellular content and capacity for DNA synthesis occur in rats suffering from neonatally induced GVHD.

GVHD was induced by injecting 40×10^6 parental strain lymph-node cells (PSLNC) on the day of birth (day 0) into the F₁ hybrid offspring of two inbred strains of rats (Fischer and DA). Newborn F₁ hybrid animals were used because they are genetically tolerant of grafts from either parent and, thus, especially susceptible to immunological attack by parental lymphocytes. Non-injected littermates or littermates injected with harmless, syngeneic F₁ hybrid LNC were used as controls. At various ages, the pups were killed and alterations in cerebellar development assessed. First, cell acquisition was estimated by measuring total cerebellar DNA content. Second, the uptake of ^3H -thymidine (^3H -TdR) into the DNA tissue fraction from the acid-soluble tissue fraction was assessed 1 h after an intraperitoneal injection of 2 μCi per g body weight. General growth of the cerebellum was measured by determining organ weight changes and total organ RNA and protein content.

The time course of GVHD effects on cerebellar maturation is characterised by a rapid appearance of changes. Thus, at the ninth postnatal day, injected animals were relatively normal by the parameters measured here. However, at postnatal day 11 cerebellar development was noticeably altered.

As seen in Table 1, cerebellar weights were affected in animals injected with PSLNC. Between days 9 and 11 there was only a 19% increase in cerebellar weight in animals with GVHD, compared with a 26% increase in controls. Subsequent to this initial change in cerebellar weight gain, cerebella from GVHD animals remained significantly smaller than those of non-injected littermates. In addition to our assessment of GVHD effects on cerebellar weights we also weighed each intact animal, its spleen and thymus, and found weight alterations similar to those previously described in animals with neonatal GVHD⁶.

In animals injected with PSLNC, both cell proliferation and RNA and protein contents were decreased compared to either non-injected controls or controls injected with syngeneic cells. This effect was proportional to changes in cerebellar weight (Table 1).

At each day including and after day 11, there was significantly less DNA, RNA and protein, indicating a marked decrease in accretion of cell number and cellular content (Table 1). There was actually less DNA at day 14 than day 13 in cerebella from GVHD animals, possibly reflecting a degree of cell death in addition to the slowed cell proliferation. Compared to controls, the uptake of ^3H -TdR into the DNA fraction from the acid-soluble fraction was less in GVHD animals at each day

measured except day 9. Labelling of the acid-soluble fraction was not significantly different in GVHD compared to controls, but there was a great difference ($P < 0.001$) in the amount of label in the DNA fraction, suggesting that although cerebellar cells in GVHD animals can take up ^3H -TdR, the mechanism for incorporating the label into DNA is altered. It is especially noteworthy that these actions on brain growth occur at a time before body wasting is evident. Animals which received syngeneic cells, that is, lymphocytes not reactive against the host, were similar to non-injected controls with respect to either cellular acquisition or cellular content.

Histological preparations showed the regular arrangement of the layers of the cerebellum to be undisturbed in 14-d-old animals with the classic symptoms of GVHD. In addition, there was no mononuclear cellular infiltration of the cerebellum, which argues against a direct, cell-mediated attack by invading lymphocytes against cerebellar cells.

The demonstrated effects of GVHD on maturing rat cerebellum may be highly significant with respect to the development of the human cerebellum, which acquires 80% of its total neuronal cell population, that is, granule cells, during the first two postnatal years⁷. Therapeutic measures, including exchange transfusion of the immunologically immature fetus *in utero*⁸, routine transfusion of infants suffering from congenital immunological deficiency syndrome⁹, and even of lymphocytes from the maternal to the infant circulatory system¹⁰ can all place infants at risk due to the induction of a GVHD syndrome. In addition, bone marrow transplantation in children with haematological disorders can result in GVHD, and affected infants often survive mild or moderate episodes of the disease¹¹. Human infants do, in fact, receive foreign lymphocytes in these instances at a time when cerebellar cells are still proliferating, but there has been no assessment of potential CNS damage due to induction of graft-versus-host disease.

The adverse actions described here of GVHD on cell proliferation in the developing rat cerebellum indicate that the CNS is a previously unrecognised site of damage during this syndrome. The mechanism by which GVHD causes damage to cerebellar growth is still obscure. No lymphocytic invasion of the cerebellar tissue was observed in affected animals, which suggests that a blood-borne factor may be involved. Alterations in cerebellar synthetic capacity could be the result of several different agents during the course of the disease; for example, hormones like cortisone¹² or factors from activated lymphocytes such as proliferation inhibitory factor.¹³ It is conceivable that the cerebellum could be affected as an innocent bystander in a similar way to other tissues such as the skin¹⁴ and intestine¹⁵ of

animals with GVHD. Our preliminary histological studies have suggested a depletion of cells in the external germinal layer, an effect quite different from that found in malnutrition¹⁶ and some hormonal alterations¹⁷. The analysis of the process by which graft-versus-host disease selectively slows growth within the proliferative cell population in the cerebellum remains a challenge to the understanding of basic growth processes in neural development, and is under investigation in our laboratory.

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Dissociation between learning and remembering in organic amnesia

IN Korsakoff's disease and certain other cases of organic amnesia there may be profound disturbance of memory with little or no other intellectual disturbance. The nature of this memory defect is controversial and in particular there is disagreement about the stage of the memory process which is impaired. There is evidence in support of three possibilities¹⁻³. Information might be inadequately acquired, maintenance of information in store might be defective or information might be inadequately retrieved from store. The results reported here suggest that inadequate acquisition is the most likely cause of the amnesia in Korsakoff's disease.

It is known that amnesic patients are inferior to controls in recognising previously seen pictures when conditions of presentation and testing are identical for both groups^{4,5}. However, when pictures are presented to Korsakoff patients for a period four to eight times longer than that given to controls, and both groups are tested 20 min later, the recognition performance of the patients is raised to that of controls⁶. We have used this effect to establish equal performance of two such groups on picture recognition after a 10 min interval and then to compare their speed of forgetting as shown by their performance after longer intervals. This obviates the difficulties that have beset previous attempts to compare the rates of forgetting of amnesic and control subjects (see ref. 7 for review).

The Korsakoff patients were seven severely amnesic males with a history of alcoholism. Four were tested at the Boston or Bedford Veterans Administration Hospitals (Massachusetts) and three were tested at the Manchester Royal Infirmary. All had a severe defect of recent memory but other cognitive

functions were intact. Their mean age was 58.4 yr and mean IQ on the WAIS scale was 104. The control group consisted of six males with no evidence of neurological or psychiatric disturbance who were ambulant inpatients on the general medical and surgical wards of the Boston VA Hospital. Their mean age was 58.8 yr and mean IQ 116.

Each subject was shown 120 slides of coloured pictures photographed from magazines (stimulus items) and asked to remember them. Recognition memory was tested 10 min, 1 d and 7 d later. In each test, a sample of 40 of the original pictures was presented, randomly interspersed with 40 new pictures (distractor items), and the subject was asked to say for each picture whether or not he had seen it previously. We aimed for 75% correct recognition by both groups after the 10 min interval. Because there was no certainty that individual subjects would achieve this level of performance after a particular exposure to the stimulus items, two sets of 240 pictures were prepared. These were all drawn from the same source and individual pictures were allocated randomly to the first or second set and, within sets, to stimulus and distractor items. The 120 stimulus items from the first set were shown to the controls for 1 s each and the range of correct responses 10 min later was 71.3-85%. The same stimulus items were presented for 4 s each to the Korsakoff patients. This enabled four of them to attain a level of performance after 10 min comparable to that of controls (78.8-83% correct). For the remaining three patients, 4 s was inadequate: their performance at this exposure was therefore treated as a calibrating experiment and was not analysed. Six hours later, these three patients were shown the second set of 120 stimulus items for 8 s each. This resulted in recognition after 10 min equal to that of the controls (72.5-80% correct). A Mann-Whitney 'U' test then confirmed that after 10 min there was no significant difference between the performance of the seven amnesic and the six control subjects ($P = 0.53$).

All subjects were tested again 1 and 7 d later, using slides from the appropriate set. The results are shown in Fig. 1. A two-way analysis of variance was carried out and this showed that the interval between exposure to stimulus items and testing (the retention interval) was the only significant source of variation ($F = 46.5$, $P < 0.001$). Neither the between-groups component nor the interaction between group and retention interval was significant. An identical pattern of results was obtained when the raw data and two kinds of transform of the data (inverse sine and logistic) were analysed. Detailed comparisons showed no significant difference between the groups for any of the three retention intervals. Furthermore, the distribution of true positive and true negative responses was virtually identical for the two groups for each retention interval (Table 1). Thus, recognition performance declines with increasing retention interval but the rate of decline does not differ between the groups. This identity of performance between the groups cannot be explained

Fig. 1 Mean % correct (TP+TN) responses (± 1 s.d.) on picture recognition test. Speed of forgetting is identical when initial performance (at 10 min) has been equated. Solid line, Korsakoff patients; dashed line, control subjects.

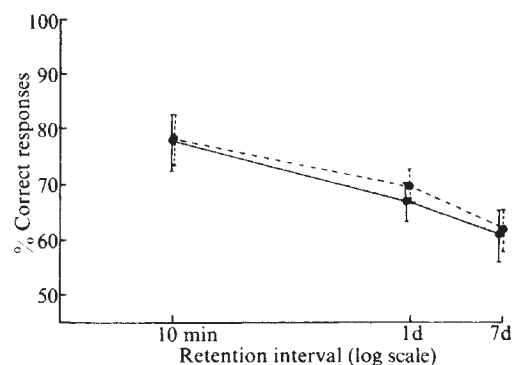


Table 1 Mean true positive (TP) and true negative (TN) responses on picture recognition test (maximum = 40)

	Retention interval					
	10 min		1 d		7 d	
	TP	TN	TP	TN	TP	TN
Korsakoff patients	26.7	35.6	21.1	32.0	19.4	29.4
Control subjects	28.5	34.2	24.2	31.6	20.5	29.2

by the somewhat different intelligence of the two groups for two reasons. (1) When Korsakoff patients and controls are strictly matched for IQ, their recognition performance is identical after a standard retention interval if the patients receive the stimulus for 4–8 times as long as do controls⁶. (2) In our experiment, the Korsakoff patients were on average somewhat less intelligent than the controls and therefore could not have compensated for amnesia with more intelligent strategies for learning and remembering. The performance of the Korsakoff patients was indistinguishable from that of the controls not only after 10 min (when performance of the two had been deliberately equalised) but also after the two longer intervals when any difference in the ability to remember should have become apparent. Clearly, it is not in their recognition performance that the groups differed, but in the time available to acquire information when the pictures were first presented.

The simplest interpretation of our results is that the amnesic defect of the Korsakoff patients resides in the initial learning of the information. This interpretation can be avoided only by assuming that the additional time for learning made available to Korsakoff patients resulted in their acquiring more information than the controls and losing this advantage within 10 min through defective retention or retrieval. However, such an advantage could not have been lost through faulty retention because this would require the curves to diverge after the 10 min test (Fig. 1) and the Korsakoff patients to perform abnormally poorly after the longer intervals. Nor could such an advantage be lost through greater susceptibility to interference from earlier memories—the most frequently advocated ‘retrieval theory’ of amnesia^{3,8–10}—such interference increases with retention interval¹¹ and accordingly the Korsakoff patients should forget faster than the controls. Again the curves should diverge.

It therefore seems very likely that the extra time provided for the Korsakoff patients was utilised to achieve a level of learning comparable with that which the controls achieved in less time. Accordingly, at least for picture recognition, the Korsakoff patients seem to have a defect in the initial learning of information but forget no faster than normal. Normal rates of forgetting in Korsakoff patients might seem paradoxical if organic amnesia is seen as inability to remember. However, apparent defects of retention or retrieval in these patients could in principle be—and, we suggest, are—secondary to a defect of initial learning.

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Mechanism for the formation of synaptic projections in the arthropod visual system

THE axons of photoreceptors in arthropods project in a precisely ordered manner from the retina to the lamina. As retina and lamina consist of two-dimensional arrays of structural units (the ommatidia and the cartridges, respectively), a particular unit of origin and a specific target unit can be defined uniquely for each axon. In a number of species, such as water fleas¹, crabs² and locusts³, all axons originating from an ommatidium terminate in the same cartridge, where they form synapses onto laminar neurones. How is a particular target cartridge selected by the reticular axons as they grow into the laminar anlage during embryogenesis? The adult pattern of projections could arise in several ways. For example, each ommatidium and each cartridge could have unique chemical labels varying with or according to their positions in their respective domains. Input and target could then be matched by a chemoaffinity mechanism such as that originally proposed by Sperry⁴ for the formation of vertebrate retinotectal connections. A second possibility is that reticular fibres do not recognise any specific labels in the laminar cells, but grow into this region in the appropriate spatio-temporal order to sequentially fill available sites in a pre-formed laminar array. In a variation of this second mechanism the lamina would be unstructured at the time of arrival of reticular axons and the axons would impose a pattern on the lamina by sequentially recruiting immature laminar neurones to form the regular array of cartridges. These possibilities have been tested by deleting ommatidia at specified developmental stages in *Daphnia magna*, a small branchiopod crustacean with a total of 22 ommatidia and 22 cartridges⁵. The observations reported here support the last hypothesis, that the order of reticular axon ingrowth determines the retino-laminar projections in this organism.

The formation of the retino-laminar projection in *Daphnia* follows a precise sequence of events which occurs during the middle stages of embryogenesis^{5,6}. This is shown schematically in Fig. 1. Reticular axonal bundles arrive in order at the midline of the laminar anlage, where they contact immature laminar neurones with which they will subsequently form cartridges and move away from the midline. In order to determine whether a bundle of reticular fibres only interacts with a specific, pre-determined group of cells, I used a UV radiation microbeam to delete ommatidia during development before their axons made such contacts. Two possible outcomes of such an experiment are shown schematically in Fig. 2. On the left, cartridges which normally correspond to the deleted ommatidia receive no reticular projections; on the right, the last cartridges that would form are the affected ones.

Lesions were made in about 40 embryos at different locations in the eye anlage, deleting 1–10 ommatidia before their fibres grew into the lamina⁷. Animals were fixed at various times after the irradiation and serially sectioned for light or electron microscopy. The analysis was in part performed with the aid of three-dimensional computer reconstructions from these sections⁸.

Some data on experimental animals analysed as adults are presented in Table 1. For every ommatidium which was completely removed in the embryo, five laminar neurones, and

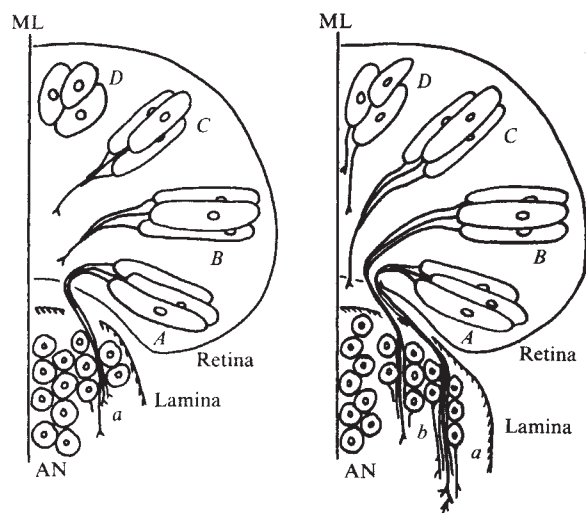


Fig. 1 Schematic diagram of a rostro-caudal cross-section of two *Daphnia* embryos; left, 30 h old; right, 33 h old. Only the eye and lamina on the right of the midline are shown. A–D, Ommatidia; a, b, cartridges. ML, midline; AN, available immature neurones. The photoreceptors in ommatidium A are the first to differentiate. They send processes along the midline into the lamina, where they encounter laminar cells without processes. Within each future ommatidium one cell matures earlier than the other seven; its growing fibre precedes or 'leads' the other seven into the lamina. The lead fibre sequentially contacts five laminar cells which then wrap around this fibre and form transient gap junctions with it^{5,6}. The cells which are contacted are always those closest to the anterior surface and the midline of the laminar anlage. As the following fibres of the bundle arrive, the laminar cells unwrap partially, form a ring around the bundle and elaborate processes which grow in parallel with the receptor fibres into the forming neuropil. After cartridge a is formed in the lamina, it moves laterally as the fibres from ommatidium B arrive. After cartridge b is formed, it also moves laterally behind cartridge a. The fibres from ommatidium C then arrive at the midline and contact five immature neurones. At 33 h, the first cartridges to form have begun to elaborate processes which grow into the future neuropil region. Synapses will appear a few hours later.

hence a cartridge, were missing in the adult. When an ommatidium was only partially deleted, however, a smaller than normal cartridge was formed. From these data it was not possible to determine conclusively which one of the cartridges was missing when an ommatidium was removed. In no case out of 17 analysed in detail were there found any areas filled by glia but devoid of neurones, or any groups of degenerating laminar neurones that would allow identification of the missing cartridges. In each case the cross-sectional area of the lamina was reduced in proportion to the number of missing structures. (It has also been shown that eye deletions strongly affect laminar structure by genetic manipulation in *Drosophila*⁹ and surgical manipulation in other species^{10,11}.)

Table 1 Numbers of reticular neurones, ommatidia, laminar neurones and cartridges in animals irradiated as early embryos and analysed as adults using 3-D computer reconstructions from serial sections

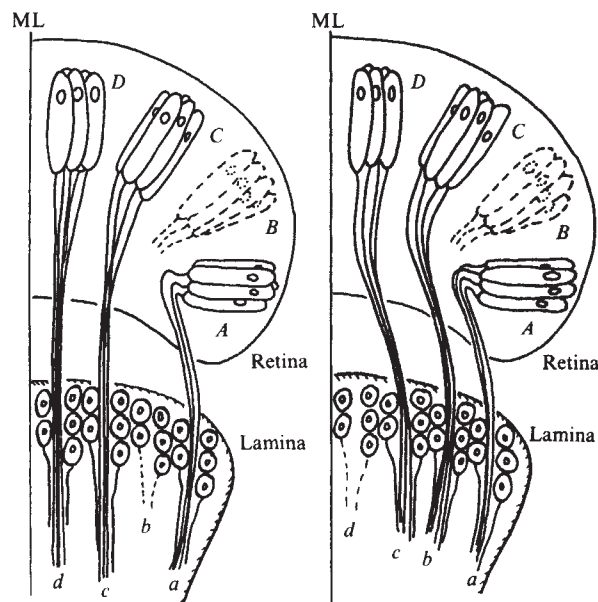
Specimen	No. of reticular neurones	No. of ommatidia*	No. of laminar neurones	No. of cartridges*
A	163	20	99	20
B	153	20	94	20
C	116	18	87	18
D	87	14	68	14
E	89	12	57	12
F	127	16	79	16
Control	176	22	110	22

* Some ommatidia and their associated cartridges have fewer than the normal complement of neurones (8 and 5, respectively) in experimental animals.

Two observations on experimental animals analysed at embryonic stages ranging from 3 to 20 h after irradiation showed that the cartridges which degenerate are the last ones to form. First, immature laminar neurones, including those that later disappear, are still to be found within the laminar anlage until some hours after all reticular fibres normally arrive at the lamina. No signs of laminar cell degeneration are found until some 10–12 h after the 38 h stage, when the last bundles grow into the lamina (see Fig. 3). Second, no group of laminar neurones without an associated bundle of reticular fibres is found away from the midline at any stage during or after the arrival of the reticular bundles. In other words, at no developmental time is there a situation like that shown in Fig. 2 (left). Instead, laminar cells that are not contacted because of the reduced number of reticular bundles are found near the midline, as diagrammed in Fig. 2 (right) (see also Fig. 3). These left-over cells fail to elaborate fibres at the time when corresponding normal groups, located symmetrically about the midline, are well differentiated.

The extra laminar cells could, in principle, be either the last group to appear, group d in Fig. 2, or group b which, as it was never contacted because ommatidium B was deleted, has remained near the midline. My observations of the temporal pattern of fibre ingrowth and of the geometry of the laminar anlage support the first alternative. The immature neurones contacted by a growing fibre bundle are always those closest to the anterior surface and the midline. Therefore, if growing bundle C is to contact laminar group c, then group b must be displaced away from the midline. Yet a group of laminar cells is never observed away from the midline without an associated reticular fibre bundle. It is conceivable, though rather unlikely because of the geometry of the developing lamina, that groups b and c permute positions if b is not contacted at a specific time. However, in no experimental animal studied at the appropriate embryonic stage was there any indication that laminar cells move from the anterior surface back toward the more posterior regions from which they originally arise. Though I cannot absolutely dismiss the possibility that group b recycles back to

Fig. 2 Two possible outcomes of the deletion of ommatidium B. (Nomenclature as in Fig. 1.) Left, if ommatidium C recognises and contacts only the laminar cells which will form cartridge c, then we should find, at some developmental stage, a group of neurones in the b position which do not receive a reticular projection. Right, if the fibres from ommatidium C recruit whatever laminar cells are available, they will contact those cells that will normally become cartridge b. The deletion of ommatidium B then would lead to the d laminar cells remaining untouched and available.



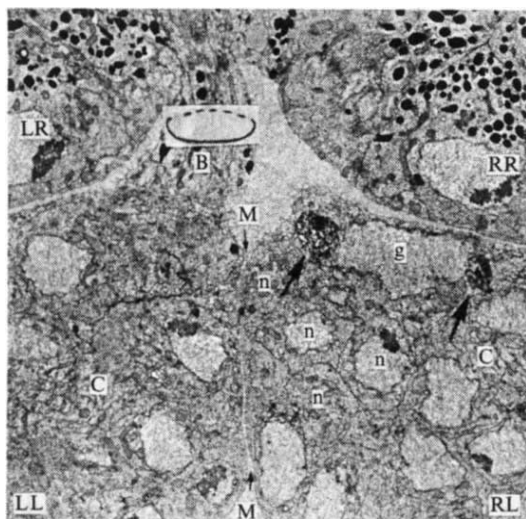


Fig. 3 Low power electron micrograph of a rostro-caudal section from a set of serial sections of an embryo irradiated on the right side at 31 h and fixed at 50 h. M, midline; LR and RR, left and right retina; LL and RL, left and right lamina; C, cartridge; B, fibre bundle travelling from LR to LL. The corresponding fibre bundle on the right was deleted by irradiation. Arrows indicate debris from degenerating cells which has been internalised by glial cell g. Immature neurones, n, that remain without an associated reticular bundle are found at this stage only on the right side of the midline. Computer reconstructions from the serial micrographs show that these immature neurones have failed to elaborate processes, unlike the neurones in corresponding positions on the left side of the midline. Note that, because of these left-over cells, cartridge C on the right is more distant from the midline than the one on the left. $\times 2,400$.

the surface region where the left-over cells are found, I believe from my observation of the temporal sequence of events that it is highly unlikely. I conclude that the extra cells are the last to arrive, that is, those that normally would have become part of the cartridge *d*.

From these observations the following developmental scheme in the *Daphnia* visual system can be formulated. As reticular fibres arrive at the laminar anlage they recruit whatever immature laminar neurones are available at that time and in that position. (This recruitment process could be mediated by signals exchanged during the early, characteristic interaction between reticular fibres and immature neurones which have been described previously⁶.) The order of synaptic projections is a consequence of the spatio-temporal sequence of ingrowth of optic fibres and no special affinity of ommatidia for specific targets is required. In animals with retinal lesions the extra laminar cells fail to differentiate because they do not receive a required signal from reticular fibres. The degeneration of laminar cells deprived of contacts provides evidence supporting cell death as the mechanism for regulating relative numbers of neurones in this system. Such a regulatory role for cell death has been previously suggested in both invertebrate¹² and vertebrate nervous systems¹³.

In conclusion, experimental evidence has been obtained which indicates that, in a structure whose parts normally mature in a spatially and temporally ordered fashion, neurones and their targets need not be individually labelled in order for appropriate synaptic projections to be achieved. Horridge³ arrived at a similar conclusion when he observed normal optokinetic responses by locusts that had regenerated eye-lamina connections following eye rotation at the second instar larval stage. However, as about two-thirds of the retina is added at later instars¹¹, it is unclear whether this result is due to normal development of the remaining retina following the graft rotation or to the regeneration of connections from the existing ommatidia. A spatio-temporal mechanism has also been suggested by Gottlieb and Cowan¹⁴ as a possible way to account for the

genesis of the pattern of synaptic connections in the dentate gyrus of the rat. In those cases where all fibres grow in and form synapses simultaneously, however, a different mechanism, such as chemoaffinity⁴ or fibre-sorting¹⁵, might be necessary.

Although sequential differentiation of photoreceptors also takes place in *Drosophila* and other insects (see ref. 16 for review), the details of the developmental interactions are not known in these species. My results on *Daphnia*, and parallels in the developmental process in various species¹⁶, suggest that mechanisms involving cell-cell contact as the signal for specific recruitment and differentiation of target cells may be generally valid in the formation of the retino-laminar system of invertebrates.

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Phase-locking in goldfish saccular nerve fibres accounts for frequency discrimination capacities

THE auditory systems of fishes have been of particular interest in the development of auditory theory because a mechanical analysis of frequency had seemed unlikely on the basis of the ear's rather undifferentiated structural organisation¹. Thus, at the level of the 8th nerve, information about stimulus frequency is thought to be coded in the temporal structure of neural activity rather than in across-fibre distributions of impulse rate²⁻⁴. However, recent neurophysiological data show that a limited peripheral frequency analysis occurs in several species⁵⁻⁷ and some workers have suggested that this may form the basis for psychophysical demonstrations of filtering⁸⁻¹⁰ and for frequency discrimination in the fish auditory system^{11,12}. Such a 'place principle' of frequency analysis and pitch perception is presently generally believed to originate in the phyletic series among the amphibia and to reach the greatest degree of elaboration with the mammals¹³. We report here an experiment which helps to resolve this question of frequency codings by evaluating the adequacy of the temporal hypothesis to account for the behaviourally measured frequency difference limens for the goldfish^{11,12}. A simple form of the temporal coding hypothesis states that the reciprocal of stimulus frequency (period) is

represented in 8th nerve fibres as the distribution of time intervals between phase-locked impulses⁴. A discrimination between two different period lengths may thus entail a decision as to whether the two resulting distributions of neural interspike intervals are the same or different. Clearly, the performance on such a discrimination would be limited by the variability of the distributions, or the accuracy with which neural impulses are phase-locked to the stimulating waveform. We demonstrate here that the neurones which phase-lock best at any given frequency show degrees of phase-locking accuracy that would be expected if the central nervous system were processing the input optimally.

The details of the method used for the neurophysiological measurements have been published previously⁷. Phase-locking variability of single saccular neurones of the goldfish was measured in response to tones presented at the frequencies and amplitudes used in behavioural measurements of frequency discrimination¹¹. Extracellular potentials were recorded from single saccular nerve fibres in 15-cm common goldfish, anaesthetised (MS-222) and immobilised (Flaxedil). Generally, saccular neurones could be classified into high frequency (HF) and low frequency (LF) types. The LF neurones showed best frequencies below 200 Hz, whereas the HF type showed best frequencies ranging between 300 and 800 Hz. Highly spontaneously active neurones showed little adaptation and were stimulated with continuous tones of up to 10 s duration. Neurones with little or no spontaneous activity showed rapid adaptation and were stimulated with 100-ms tone bursts, repeated at 2 Hz.

Neurones could be 'held' from seconds to over an hour, with the LF type usually lasting longer and exhibiting larger spikes (5–40 mV) than the HF type (0.5–20 mV). When a neurone was encountered, it was stimulated with a set of frequencies at sound pressure levels 35 dB above behavioural threshold¹⁴ until it was 'lost' or until the stimulus set was exhausted. For each signal, a 10-min period histogram¹⁵ was obtained from which measures of phase-locking variability were made. Figure 1 shows the period histograms obtained for four representative neurones

Fig. 1 Period histograms for four representative neurones, 204(a), 205(b), 209(c) and 216(d), from one animal for 1 s of sinusoidal stimulation at the frequencies indicated. Sound pressure levels are 35 dB above behavioural threshold¹⁴. The number associated with each histogram is the standard deviation of the distribution in ms.

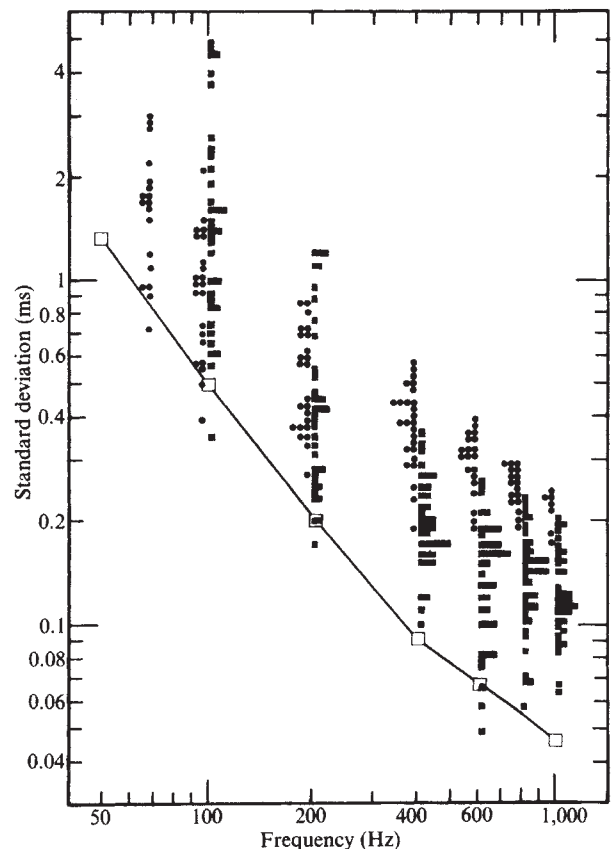
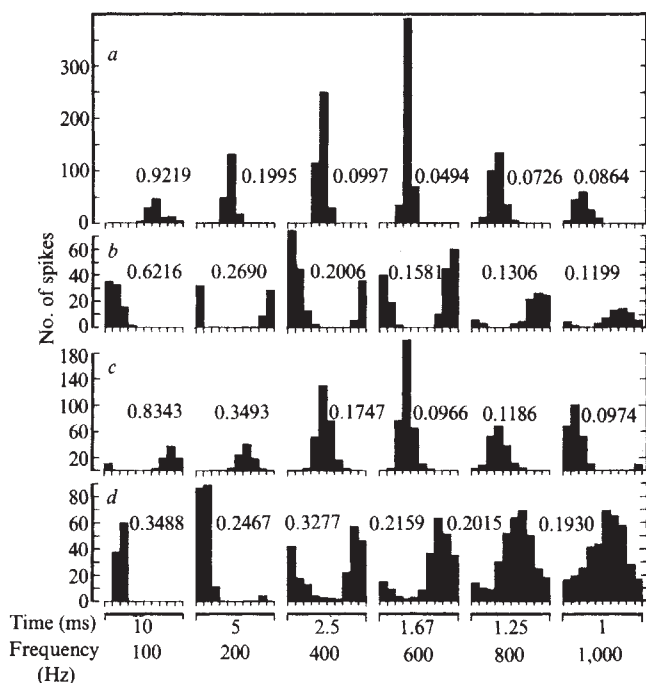


Fig. 2 The standard deviations (in ms) of period histograms for 45 high-frequency neurones (■) and 22 low-frequency neurones (●) to sinusoidal stimulation at 35 dB above behavioural threshold¹⁴ from nine animals. The line connects the mean just-noticeable differences in stimulus period (in ms) measured psychophysically for four animals in a previous study¹¹.

from a single animal. From each histogram, average discharge rate, the location of the mean (phase angle) and the coefficient of synchronisation (R) were calculated¹⁶. The coefficient R was then transformed into a standard deviation value in ms (ref. 17). These values are plotted as a function of frequency in Fig. 2 for 63 of the most sensitive neurones encountered in this study (nine animals). Also plotted in Fig. 2 are the mean just-discriminable differences in period length (ΔP) for four goldfish tested in similar stimulus conditions in a previous study¹¹. The behavioural ΔP values generally fall within the range of the smallest period histogram standard deviation values obtained at each frequency.

A simple temporal coding hypothesis holds that stimulus period lengths are estimated by the measurement of the temporal intervals between nerve impulses¹⁸. The discrimination problem may be viewed as a decision as to whether two samples of neural interspike intervals are drawn from the same, or two different underlying distributions. Assuming that the means of these hypothetical distributions are equal to the periods of the two signals to be discriminated, and that the distributions' variance is completely determined by the phase-locking variance, then we would expect threshold-like behaviour to occur when the difference between the means (ΔP) is approximately equal to the distributions' standard deviation (sd), that is, when $d' = \Delta P/sd = 1$ (ref. 19). The results show that this is indeed the case for the smallest sd values at each of the frequencies studied. Thus, peripheral phase-locking variance accounts well for the errors made in behavioural frequency discrimination, with the assumption that the decisions are based on the small percentage of neurones transmitting the best information.

A similar analysis was carried out by Mountcastle *et al.*¹⁸ for cutaneous flutter vibration discrimination. Human vibratory frequency discrimination performance was compared with the standard deviations of period histograms for somatosensory cortical neurones in the monkey. For vibratory frequencies between 5 and 200 Hz, the behavioural estimates of the *sds* of the underlying sensory distributions fell within the range of the smallest neural *sds*. These results for the cutaneous system are also similar to the present results in that both neural and behavioural measures of error decline approximately linearly with frequency (with unity slope in log-log coordinates), and in that the actual values of the cutaneous *sds* are only about twice as large as those of the goldfish auditory system.

Although the present results show that a simple temporal coding hypothesis accounts quite well for the behavioural capacities for frequency discrimination in the goldfish, possible alternative hypotheses are not ruled out. Further experiments are required in which phase-locking variance may be manipulated; for example, through the introduction of temporal 'jitter' in the stimulus waveform, or through the use of aperiodic stimuli such as amplitude-modulated noise²⁰. The nature of central neural mechanisms which decode the temporal input patterns remains to be explored. The goldfish seems to be a valuable preparation for combined behavioural and neurophysiological approaches to the coding of temporal patterns by nervous systems.

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Sinus node extracellular potassium transients following vagal stimulation

SEVERAL investigations have suggested a role for K^+ in the generation of the transmembrane potential during spontaneous rhythm^{1,2} and following vagal inhibition^{3,4} of the sinoatrial node (SA node) of the heart⁵. Voltage-clamp studies^{1,2} in the rabbit SA node have shown a voltage- and time-dependent increase in the outward current on depolarisation and a slow decay of the outward current on repolarisation to the holding potential. The hyperpolarisation and slowing of automaticity following vagal stimulation or acetylcholine (ACh) application is believed to be due to an increase in potassium permeability^{3,4}. The purpose of the present study was to use K^+ -sensitive microelectrodes^{6,7} to examine the time course and the magnitude of K^+ efflux and

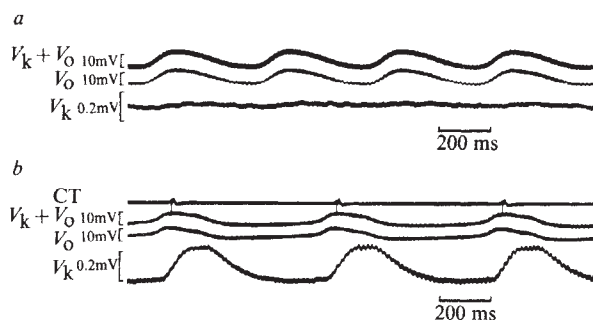


Fig. 1 a, The response of both barrels of a double-barrelled K^+ -sensitive microelectrode to skewed sine-wave calibrating pulses of similar time course and magnitude to extracellularly measured action potentials. Selective barrel, $V_K + V_0$; reference barrel, V_0 . V_K represents the differential output showing common mode rejection at this level of resolution. b, The output of both barrels with the double-barrelled K^+ -sensitive microelectrode in the extracellular space of the SA node. Selective barrel, $V_K + V_0$; reference barrel, V_0 . The differential trace, V_K , now shows periodic fluctuations with the same periodicity as the crista terminalis electrogram (CT) and extracellular potential fluctuations (V_0). V_K represents beat-to-beat alterations in $[K^+]_o$ and is uncontaminated by common mode rejection artefacts (see a above). In the V_K record 0.2 mV is equivalent to 0.034 mM potassium.

extracellular accumulation in the isolated rabbit SA node during spontaneous rhythm and following vagal stimulation. Our results confirm that K^+ permeability of the sinoatrial pacemaker cell is increased following vagal stimulation and demonstrate K^+ accumulation in the extracellular space for several seconds. Beat-to-beat extracellular potassium transients are linked to the repolarisation process as well as to diastolic depolarisation.

Experiments were carried out on rabbits (1-3 kg) anaesthetised with 10 mg per kg intravenous sodium pentobarbital. The hearts were removed and placed in Tyrode's solution, gassed with 95% oxygen and 5% CO_2 , and maintained at 37 °C; 1.0 mg l^{-1} propranolol was added to the Tyrode's to eliminate β -adrenergic influences. The atrium was opened according to the dissection of Paes de Carvalho⁸. Vagal stimulation was accomplished by a previously described⁹ modification of the technique of Vincenzi and West¹⁰. Bipolar silver electrodes were placed on the endothelial surface of the superior vena cava in the region traversed by vagal fibres innervating the sinus node area¹¹. The vagal stimuli were delivered as a train of 4-ms square-wave pulses at 100 Hz for 100-300 ms. Silver bipolar electrodes were used to record from the surface of the crista terminalis (CT) and right atrial muscle. Standard 3 M KCl-filled microelectrodes were used to record transmembrane potentials from sinus node fibres.

Double-barrelled K^+ -sensitive microelectrodes were prepared by filling the tip of one barrel of a double-barrelled glass micropipette, pretreated with a siliconising agent, with K^+ -selective resin (Corning liquid resin no. 477317 with $K^+ : Na^+$ selectivity of approximately 60:1) (refs 6, 12). The selective barrel was then backfilled to the top of the resin column with 100 mM KCl. The other barrel was backfilled with normal Tyrode's solution. K^+ electrodes with tips of 3-4 μm had a resistance of about 1,000 M Ω and an electrical response time of 15-20 ms. The chemical response time of the electrode to variation of K^+ was found to be faster¹². Selectivity values were measured before and after experiments with a series of calibrating solutions with potassium concentrations (mM) of 2.7, 10, 20, 50 and 100. Signals from the K^+ -selective barrel and the reference barrel were independently amplified by high-input impedance amplifiers ($1.5 \times 10^{12} \Omega$) with capacitance compensation. The amplified signals were differentially subtracted by a third amplifier (Bloom). Thus, we were able to obtain the potassium potential (V_K) free of the extracellular potential (V_0) fluctuations which were common to both barrels (Fig. 1a).

Records were simultaneously displayed on a Tektronix 5103 oscilloscope and stored on an eight-channel Hewlett Packard instrumentation tape recorder 3968A.

During advancement of the K^+ electrode into the extracellular space of the SA node, occasional large transient positive deflections on the K^+ barrel were seen. These were probably the result of the electrode rupturing cell membranes and exposing the electrode tip to the high intracellular potassium concentration. Action potentials were occasionally recorded with the double-barrelled electrode. The K^+ electrode was manipulated and either pulled back or advanced through the cell until a location was found where potential fluctuations common to both barrels were less than 10 mV. Time was allowed for elevated extracellular potassium values associated with membrane rupture to stabilise to values which corresponded to the approximate value of K^+ in the perfusate (2.7 mM).

At many of these K^+ electrode positions, beat-to-beat fluctuations in extracellular potassium were recorded (Fig. 1b). These fluctuations had the same periodicity as the spontaneous rhythm as determined by the intracellular SAN recordings and the CT electrogram (Fig. 2). The extracellular potassium concentration ($[K^+]_o$) increased gradually during phase 0 depolarisation, reaching a peak change in concentration of approximately 0.05 mM which corresponded to the middle of phase 3 repolarisation. It then subsided rapidly during the last half of phase 3 repolarisation, and the beginning of phase 4 diastolic depolarisation. There was a slower decay of potassium concentration throughout phase 4 diastolic depolarisation. The maximum peak change in K^+ concentration fluctuation in these extracellular measurements was 0.07 mM.

While continuing to monitor beat-to-beat extracellular K^+ fluctuations, vagal stimulation was applied. This was accompanied by characteristic changes in the intracellular potential response of the SA node. Initially, there was a hyperpolarisation of the maximum resting membrane potential, a decrease in the slope of diastolic depolarisation and a shortening of the action potential duration. Simultaneous $[K^+]_o$ measurements following vagal stimulation showed elevations of extracellular K^+ in the SA node of up to 0.2 mM. Following the peak accumulation, $[K^+]_o$ returned to baseline over a period of 8–13 s (Fig. 3). In some cases, $[K^+]_o$ values fell below previous baseline activity before returning to its original resting level. This potassium undershoot has previously been described by Kunze after a

period of rapid pacing in atrial muscle¹³ and by Kriz *et al.* in cat spinal cord after peripheral tetanic stimulation¹⁴. Both authors suggest that this subnormal phase of $[K^+]_o$ reflects active processes redistributing K^+ from the extracellular space.

Because of the large amount of connective tissue in the SA node^{1,2,15}, beat-to-beat fluctuations were not seen for all electrode locations⁷. In addition, in some locations where a vagus-induced $[K^+]_o$ increase is seen, beat-to-beat fluctuations are either small or below the resolution of our electrode. Furthermore, it is known that cells in the mammalian SA node are tightly packed⁵; at the point of apposition of two cellular membranes, the extracellular space takes the form of 100–150-Å-wide intercellular clefts¹⁶. The tip of the double-barrelled K^+ electrode is approximately 3–4 μ m outside diameter. Therefore, the K^+ electrode tip must rest in an artificially distorted dead space¹⁷. Thus, the efflux into the extracellular space where the electrode tip rests is substantially diluted compared with the normal cleft space. For a prolonged change in the extracellular K^+ activity of the tissue, the dead space would equilibrate with the normal extracellular concentration. However, for short-term fluctuations of K^+ activity in the intercellular cleft, the response measured by the K^+ electrode would be considerably smaller than the actual intercellular changes.

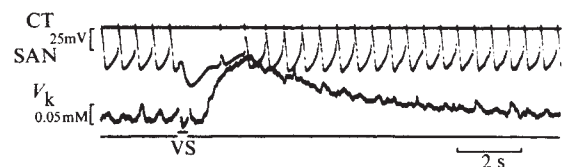


Fig. 3 Accumulation of potassium in the extracellular space of the sinoatrial node after vagal stimulation measured by a double-barrelled K^+ -sensitive microelectrode. CT, bipolar surface electrogram of the crista terminalis. SAN, transmembrane potential recording from sinus node fibre. Following vagal stimulation (VS), note the hyperpolarisation of the maximum resting membrane potential, decrease in the slope of diastolic depolarisation and shortening of the action potential duration. These events are coincident with the accumulation of K^+ in the extracellular space (V_K). Zero transmembrane potential is indicated by the upper edge of the bracket of the top calibration. 2.7 mM $[K^+]_o$ is indicated by the lower edge of the bracket of the bottom calibration.

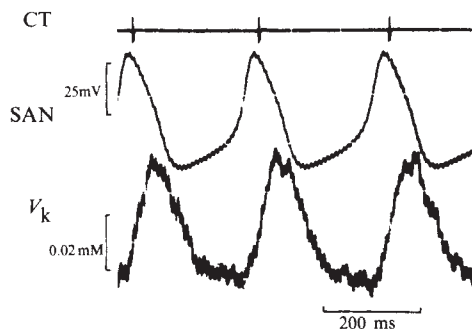


Fig. 2 Beat-to-beat extracellular potassium concentration fluctuations in the rabbit SA node during spontaneous activity measured by a double-barrelled K^+ -sensitive microelectrode. Bipolar surface electrogram of the crista terminalis at an area shown to be activated earliest during spontaneous sino-atrial conduction (CT). Transmembrane potential recording from sinus node fibre (SAN). Simultaneous potassium response was measured by subtraction of the reference barrel from the K^+ -selective barrel (V_K). As the reference barrel has been used to compensate for the electrical artefacts in the extracellular space, V_K represents the response of the K^+ -selective barrel only to changes in the K^+ concentration. The K^+ electrode is nearly linear in its response to $[K^+]_o$, for small accumulations seen during single action potentials. Zero transmembrane potential is indicated by the upper edge of the bracket of the top calibration. 2.7 mM $[K^+]_o$ is indicated by the lower edge of the bracket of the bottom calibration.

In the normal physiological range of extracellular potassium concentration, potassium-sensitive electrodes have been reported to show sensitivity to Ach fluctuations. (1 mM increase in K^+ is equivalent to the addition of 5×10^{-7} M Ach; see ref. 18.) To determine whether vagally induced release of Ach was affecting the K^+ electrode response, we added atropine to our experimental perfusate (0.2 to 1 mg l⁻¹). Atropine blocks the post-synaptic effect of Ach but not its release. We saw no vagally induced K^+ electrode response in the presence of atropine at concentrations sufficient to eliminate vagally induced rate responses. However, beat-to-beat K^+ changes were still observed.

Thus, we have confirmed the belief that the potassium permeability of the SA node pacemaker cell is increased following vagal stimulation. Extracellular potassium accumulation associated with vagal stimulation persists for several seconds and one must consider the possibility that this may contribute to membrane potential and rhythm changes after this intervention. Our direct measurement of beat-to-beat extracellular K^+ changes support the previously described observations made from voltage-clamp experiments. Beat-to-beat $[K^+]_o$ transients seem to be linked to the repolarisation process as well as to the automatic phase 4 diastolic depolarisation of the sino-auricular node of the isolated rabbit heart. This technique may thus be useful in studying the behaviour and reaction of the K^+ currents of the SA node in various conditions.

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Immunochemical demonstration of reversible reduction in choline acetyltransferase concentration in rat hypoglossal nucleus after hypoglossal nerve transection

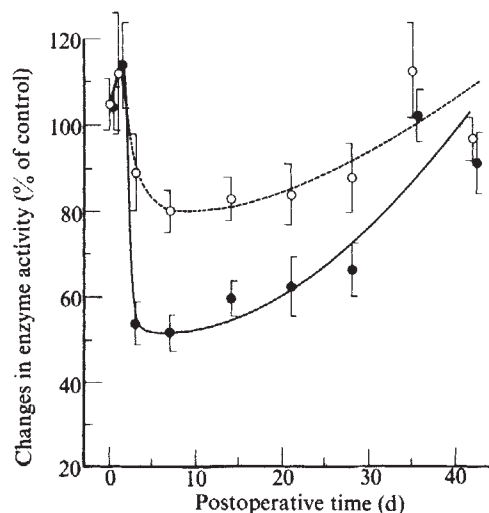
STUDIES of the cellular reaction of peripheral and central catecholaminergic neurones to axonal injury (the retrograde reaction and axon response) have demonstrated that such lesions elicit a prolonged reduction within the parent cell body in the activities and amounts of the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH) and, in noradrenergic neurones, dopamine- β -hydroxylase (DBH)¹⁻³. The reduction in enzyme protein, usually reversible, lasts for several weeks and is apparently due to a decrease in the rate of enzyme biosynthesis⁴. It has therefore been proposed that a reduction in amount and activity of neurotransmitter biosynthetic enzymes may be a biochemical indicator of the retrograde reaction³. However, it remains to be established if a reversible diminution of neurotransmitter biosynthetic enzyme occurs during the retrograde reaction in neurones which synthesise neurotransmitters other than catecholamines. This is suggested from studies of the retrograde reaction in hypoglossal neurones, cholinergic motor neurones which innervate the tongue through the hypoglossal nerve. During the retrograde reaction elicited by lesions of the hypoglossal nerve, the activities of choline acetyltransferase (CAT), the enzyme synthesising acetylcholine, and acetylcholinesterase (AChE), the enzyme degrading it, are markedly reduced⁵⁻⁷. If the proximal stump of the lesioned nerve is allowed to regenerate and reestablish synaptic contact, AChE activity within the hypoglossal nucleus rapidly reappears⁷. It is, however, unknown whether the reduction in CAT activity is reversible and, because of a lack of specific, inhibiting antibody to CAT, whether the reduction is due to a decrease of enzyme molecules. We report here that the reduction in CAT in hypoglossal neurones elicited by transection of the hypoglossal nerve

is reversible and also, by use of a specific antibody, that it is due to a decrease in the amount of CAT enzyme protein.

All studies were carried out on adult, male Sprague-Dawley rats weighing 175-225 g. While the animals were anaesthetised with 3% fluothane in 100% O₂ blown over the nose, the hypoglossal nerve was isolated unilaterally, distal to the cricoid cartilage and the major bifurcation of the nerve. A 2-3-mm segment was excised, with special care taken to include both branches. At various days after surgery the animals were killed by cervical dislocation, and the brain was removed and dissected on cracked ice. The right and left hypoglossal nuclei were removed as a block which included parts of the dorsal motor nucleus of the vagus and the nucleus tractus solitarius, and homogenised in 50 mM potassium phosphate buffer, pH 6.8, containing 1 mM sodium EDTA, 200 mM sodium chloride and 0.4% v/v Triton X-100. CAT was assayed by the radiochemical method of Schrier and Shuster⁸, and AChE was assayed by the method of Wilson *et al.*⁹. The identity of the cholinesterase activity as true acetylcholinesterase was confirmed by the observation of 90% inhibition of cholinesterase activity by 2×10^{-7} M BW 284 C51, a specific inhibitor of true acetylcholinesterase.

Antibodies to CAT were prepared by methods described in detail elsewhere^{10,11}. In summary, the enzyme was isolated from rat caudate nuclei and first partially purified by Sepharose 4B and DEAE-cellulose column chromatography, then subjected to polyacrylamide gel electrophoresis. The single active protein band was cut from the gel, suspended in 0.9% NaCl solution, thoroughly mixed with complete Freund's adjuvant, and injected subcutaneously into rabbits every 2 weeks for a total of four treatments. The serum was collected one week after the last injection. The antibody to CAT was adjudged specific by demonstrating that it formed a single precipitin line when run by immunoelectrophoresis against crude and partially purified CAT from rat caudate nucleus and that the antibody inhibited only the activity of CAT in purified form or in brain homogenates.

Fig. 1 Time course of changes in activities of CAT (closed circles) and AChE (open circles) in the ipsilateral hypoglossal nucleus following unilateral lesion of the hypoglossal nerve. Enzyme activity is expressed as % of mean activity in the contralateral control hypoglossal nucleus. Neither the activities of AChE nor CAT changed in the control hypoglossal nucleus throughout the time course studied. Each point represents the mean $\pm$ s.e.m. of 6-8 animals. The specific activities of CAT and AChE in the left and right hypoglossal nuclei of control (unoperated) rats were as follows: CAT (left/right), $80 \pm 7/74 \pm 7$ nM per mg protein per 10 min; AChE (left/right) $1.9 \pm 0.1/1.9 \pm 0.1$ μ M per mg protein per 10 min.



Immunotitration was carried out by adding increasing amounts of antiserum to CAT to 80- μ l aliquots of appropriately diluted supernatants of rat hypoglossal nucleus homogenates. Aliquots of 1:1 preimmune serum diluted with distilled water were added to each sample to bring the final volume to 90 μ l. The mixture was then agitated and allowed to stand for 1 h at room temperature with occasional shaking, incubated overnight at 4 °C, and centrifuged at 9,150g for 10 min. A 75- μ l aliquot of the supernatant was removed for assay of residual CAT activity.

Other rats subjected to hypoglossal nerve lesion were perfused with 10% formalin, the brains were removed, further fixed in Bouin's solution and embedded in paraffin. Ten- μ m sections were made through the hypoglossal nucleus, stained with cresyl violet and then examined by light microscopy.

Figure 1 shows changes in the activity of CAT in the hypoglossal nucleus at different days after hypoglossal nerve section. No change was observed after 24 h, but by 3 d the activity had fallen to 50% of control. Five weeks after the lesion, activity had returned to control levels. CAT activity did not change in the contralateral hypoglossal nucleus. Hypoglossal nerve section also produced reversible changes in AChE activity, similar in time course to but smaller than that of CAT (Fig. 1), confirming, in part, the findings of others^{6,7}.

To determine whether the transient reduction in CAT activity in the hypoglossal nucleus after hypoglossal nerve section was due to a decrease in specific enzyme protein or to an inactivation of pre-existent enzyme molecules, the degree of inhibition by antibody to CAT activity in homogenates of control hypoglossal nuclei was compared with that of nuclei whose hypoglossal nerve had been sectioned 7 d previously. The results of a typical experiment are shown in Fig. 2. As increasing amounts of antiserum were added to a fixed quantity of homogenate, CAT activity in the supernatant decreased. The slopes and equivalence points (that is, that quantity of antibody which just saturates the available antigen) of immunotitration curves for both experimental and control hypoglossal nuclei were quite similar. These results indicate that the reduction in CAT activity in the hypoglossal nucleus following hypoglossal nerve section is due to a reduction in the concentration of enzyme protein.

During the retrograde reaction, minimal changes of chromatolysis were observed in the affected hypoglossal nucleus. These changes consisted of a slight increase in nucleolar size and darkening of chromatin. No loss of neurones was noted based on careful counts of neurones in randomly selected sections through the nucleus.

The present study shows that the reduction in CAT activity in the hypoglossal nucleus during the retrograde reaction is entirely reversible. The immunotitration studies demonstrate that the reduction in perikaryonal CAT activity following hypoglossal nerve lesion is a result of a diminution in the quantity of CAT protein rather than inactivation of the enzyme. This reversible reduction in enzyme protein may be due to either a reduction in CAT synthesis or an increase in the rate of degradation of the enzyme. The observation that the reversible reduction in DBH which occurs in central noradrenergic neurones is entirely due to a diminished rate of synthesis⁴ suggests a reduction of synthesis of this specific protein. It is intriguing that the time courses of the reversible reductions in AChE and CAT activities in the hypoglossal nucleus following axotomy are so similar. Many studies suggest that AChE is transported along the axon at a very rapid rate, whereas CAT is transported at a predominantly slow rate⁵. Despite these apparent differences in axonal transport rates, our data suggest that the two enzymes may have similar control mechanisms in the cell body.

Thus, like noradrenergic neurones arising in the locus coeruleus³, and central dopaminergic neurones¹², injury to axons of the hypoglossal nerve, a cholinergic motor nerve, results in a reversible reduction in the capacity of the neurone to accumulate the enzyme which catalyses the biosynthesis of the neurotransmitter as well as the one degrading it. However, others have shown that in the hypoglossal nucleus during the period of

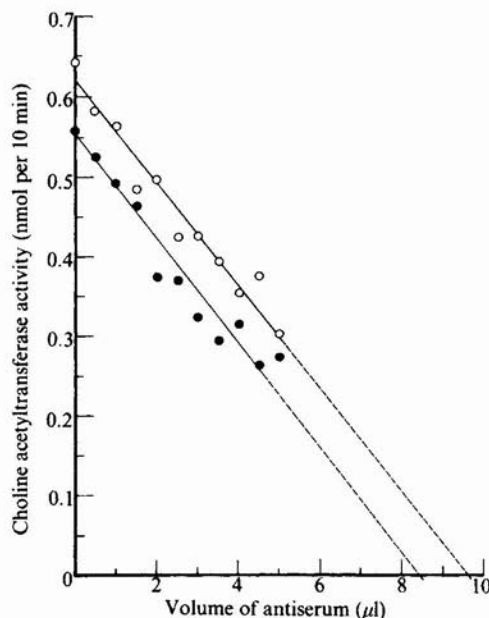


Fig. 2 Immunochemical titration of choline acetyltransferase in the hypoglossal nucleus from control (open circles) and experimental (closed circles) rats. The hypoglossal nerve ipsilateral to the experimental hypoglossal nucleus had been sectioned 7 d earlier. The homogenate of the control nucleus was diluted 1:2 so that the enzyme activity per unit volume was approximately equal in the experimental and control preparations. Note that the slopes and equivalence points are nearly identical. Linear regression analysis of each curve produced the following equations: control, $y = -0.065x + 0.621$; experimental, $y = -0.066x + 0.553$.

reduction in AChE and CAT activities there is an increase in the rate of incorporation of labelled amino acid into protein¹³ as well as an increase in the total protein content per cell¹⁴. Thus, the reduction in neurotransmitter-metabolising enzymes in cholinergic cell bodies is probably highly selective, involving a very small percentage of the total amount of protein synthesised in the cell body. These results and those of previous studies on central catecholaminergic neurones suggest that a reversible reduction in transmitter-synthesis capacity in the cell body may be a sensitive and specific indicator of axonal damage. It seems likely that during the 2–4-week period following axotomy the first priority of the neurone is to regenerate its axons and in the case of peripheral neurones like the hypoglossal, to re-establish synaptic contacts. During this recovery phase, protein synthesis associated with regeneration seems to establish priority over protein synthesis subserving neurotransmission.

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Membrane specialisations between demyelinated axons and astroglia in chronic EAE lesions and multiple sclerosis plaques

AXOLEMMAL specialisations are known to exist along myelinated fibres in the adult vertebrate central nervous system (CNS), particularly at nodes, between the axon and the oligodendrocyte, and at presynaptic sites¹. However, modified regions of axonal membrane have not been described in relation to astroglial cells. In demyelinated lesions in the CNS, it has always been assumed that the naked segment of an axon, although intimately invested by glial scar tissue, shares no junctional relationships with its scarring environment. This report documents for the first time membrane specialisations between naked CNS axons and fibrous astroglial cell processes, developing subsequent to demyelination. These structures may have physiological implications.

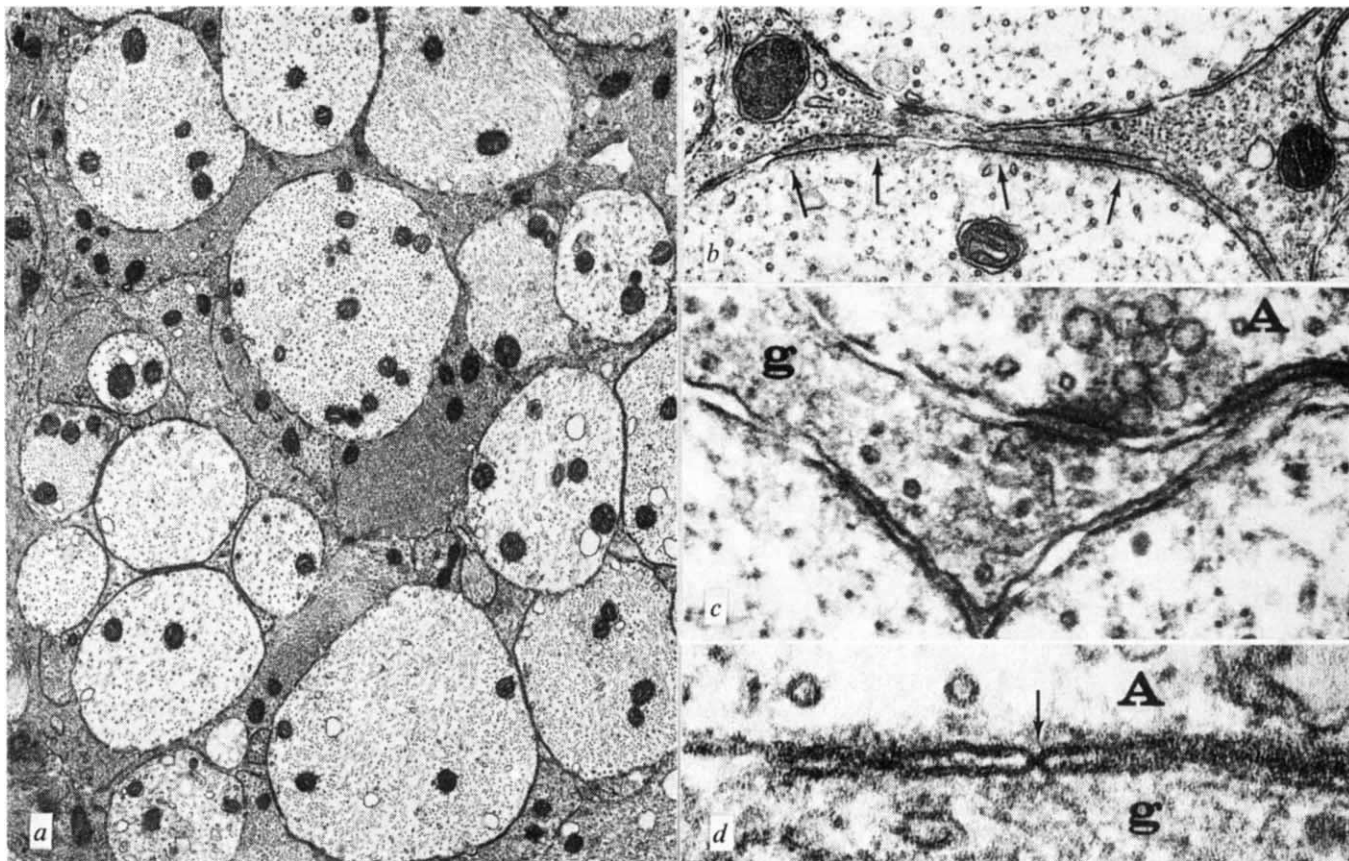
Spinal cord tissue from two demyelinating conditions have been examined. First, from strain 13 guinea pigs affected with chronic relapsing experimental allergic encephalomyelitis (EAE)², and second, from a case of chronic multiple sclerosis (MS), the paradigm of the human CNS demyelinating diseases³.

Chronic relapsing EAE was induced by sensitisation of juvenile strain 13 guinea pigs with isogeneic spinal cord tissue in complete Freund's adjuvant². Typically, animals developed delayed onset neurological signs (incontinence, paraparesis, and

so on) some 8–12 weeks post-inoculation (PI). These signs remitted, albeit incompletely, and were usually followed by several relapses. This clinical course is highly reminiscent of that of MS, for which the disease serves as a major experimental analogue. In the present study, five animals have been studied. One was sampled at the first onset of signs (10 weeks PI), three after a single relapse (12–24 weeks PI), and one after two relapses (20 weeks PI). Slices of spinal cord from L₆, L₇ and S₁ were obtained and processed for ultrastructural study after whole body perfusion with 5% glutaraldehyde buffered with PO₄, osmication, dehydration and embedding in Epon⁴. Human CNS tissue was obtained at autopsy from a patient who had suffered from chronic relapsing MS for more than 20 yr, the autopsy being carried out some 30 min post-mortem. In the lumbar cord at L₃, a large demyelinated plaque was grossly visible in the anterior column. Thin transverse slices across this lesion were taken and immersed in glutaraldehyde for 60 min and then processed as above for electron microscopy.

Demyelinated plaques of different ages were common in spinal cord tissue of the guinea pig. They were usually subpial and displayed varying degrees of developing disease according to the frequency of relapses and recurrent inflammatory activity. Lesions were occasionally grossly visible. Chronically demyelinated plaques consisted of longitudinally arranged naked axons invested by fibrous astroglial tissue (Fig. 1a). Axons were readily distinguishable from astroglial cell processes on the basis of cross-bridged neurofilaments, abundant microtubules and a circular profile^{1,5}. Astroglial processes were flattened, contained a preponderance of tightly packed filaments (not cross-bridged), fewer microtubules, and had a tendency to invest axons in an

Fig. 1 *a*, Low power electron micrograph showing a group of transversely sectioned chronically demyelinated axons from a subpial lesion in the lateral column of a spinal cord at L₇. Note how the axons are intimately embedded in a matrix of fibrous astroglial processes. Chronic EAE, 16 weeks PI, one relapse. $\times 12,000$. *b*, Higher magnification of a demyelinated axon from a dorsal column lesion at L₇ shows regional enhancement of the axolemma due to thickening of the membrane and an associated subaxolemmal coating of granular material. In two places (arrows) corresponding thickening of the parallel arranged membrane of an investing astroglial cell process is shown, between which a cleft containing electron-dense material is present. Chronic EAE, 20 weeks PI, two relapses. $\times 35,000$. *c*, An astroglial process (g) separates three naked axons, between one of which (A) a synapse-like junction is seen. Note the vesicles in the axoplasm of the affected axon (A). Same preparation as Fig. 2. $\times 100,000$. *d*, The axolemma of a demyelinated axon (A) above appears to form a fenestra (arrow) with the membrane of an astroglial cell process (g). Same preparation as Fig. 2. $\times 200,000$.



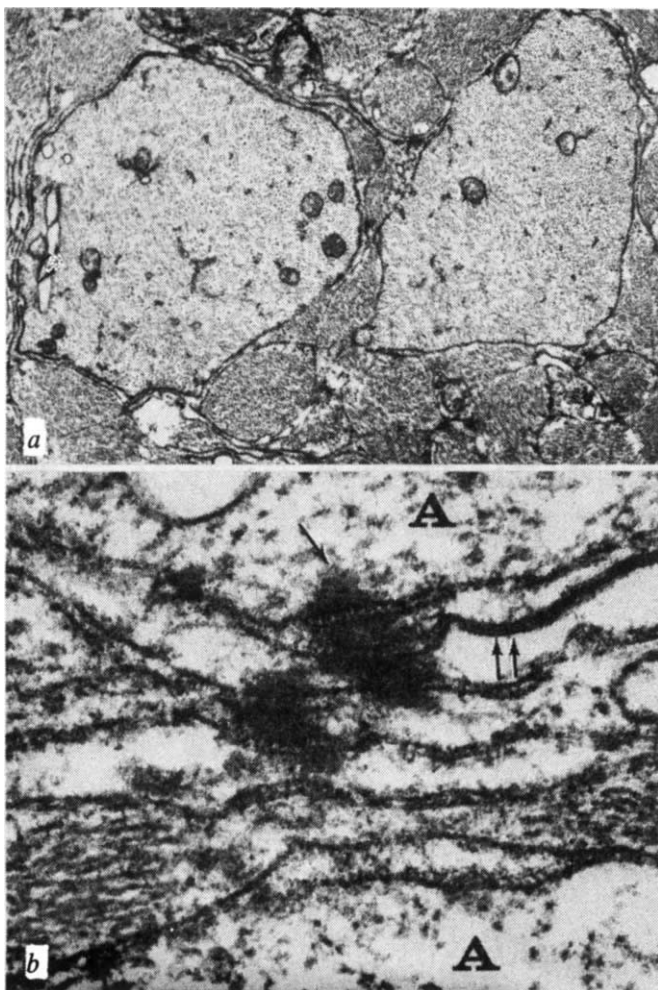


Fig. 2 *a*, Low power view of a chronic MS plaque in an anterior column of the spinal cord at L₃. Note the intense glial scarring and the two demyelinated axons in transverse section. Chronic MS, over 20 yr duration, multiple relapses. $\times 12,000$. *b*, Detail from the above chronic MS plaque showing two axons (A) above and below, one of which displays a small desmosome-like junction which contacts others traversing several interposing fibrous astroglial cell processes. A gap junction is also seen (double arrows). $\times 60,000$.

haphazard fashion. Closer examination of the naked axons revealed regional densifications of the axon membrane on the axoplasmic side of which a narrow zone of osmiophilic granular enhancement was associated (Fig. 1*b*). Not infrequently, these specialised regions of axolemma apposed a similar region of densification on the investing astroglial membrane, and between the two, a 20–40 nm cleft within which a floccular substance could be discerned. These axon–astroglial membrane specialisations were very common (occurring in more than 60% of affected axons) and were present in all chronically demyelinated EAE lesions. They were not seen in normally myelinated regions, remyelinated zones, or in areas of recent demyelination. On two occasions, axons were seen to possess primitive ‘synapse-like’ junctions with investing astrocytic processes (Fig. 1*c*). These consisted of small desmosome-like structures in association with which were 60–80 nm diameter vesicles in the juxtaposing axoplasm. In addition, three axons were seen to possess fenestrae between adjacent astroglial cell processes. These occurred along zones of specialised axolemma where the membranes of the axon and the astrocyte fused to form a single unit membrane (Fig. 1*d*). Whether or not these fenestrae represented artefacts of sectioning remains to be elucidated by goniometer stage techniques (tilting). Elsewhere throughout the EAE plaques, a variety of junctional complexes was encountered between astroglial cell processes, particularly desmosomes, and gap and punctate junctions.

In the examined MS plaque, demyelinated axons were tightly packed in an intense fibrous astroglial matrix (Fig. 2*a*). Membrane imperfections due to the poorer fixation conditions were apparent. In contrast to the EAE material, extensive regions of membrane specialisation between axolemmae and adjacent astrocytes were not seen. Instead, more than 30% of the axons examined were seen to contact astroglia by means of small desmosomal junctions—regions of membrane some 150–200 nm in extent beneath which were broad zones of electron dense granular material. Sometimes, these complexes occurred in short series from an axon across several investing glial cell processes (Fig. 2*b*). In addition, many naked axons showed extensive subaxolemmal thickening, typical of that seen at nodes¹. Synapse-like arrangements were not encountered between axons and astroglia. Gap junctions (Fig. 2*b*) and small desmosomes were common between astroglial cell processes.

The present observations of membrane specialisations and possible junctional complexes between demyelinated axons and scarring glial cell processes in the adult CNS are unprecedented in neuropathology. However, somewhat similar membrane specialisations have been described previously in the spinal cord of the mouse during development, and have been termed puncta adhaerentia (*sic*) and axoglia synapses⁶. These connections represented a transient phenomenon and were not present after embryonic day 15. Their appearance was speculated “to reflect errors in development”, although their disappearance might have been linked to the development of more specific mechanisms. These unusual thickenings might represent transient phenomena or biological errors since they do not occur along normal or remyelinated fibres. The MS plaque under study was probably many years old (compared with the 2–6 months of the EAE plaques), and the areas of membrane specialisation may have undergone some further modification with time.

That these abnormal membrane relationships between axons and glia are the result of prolonged absence of myelin seems incontrovertible and they probably play some supportive role for the axon. However, whether they impart a compensatory property on the already impaired nerve fibre conduction remains to be established. In view of the frequent lack of correlation between clinical status and the extent of damage in the demyelinated CNS, the possibility that scarring astrocytes somehow unify naked fibres and are capable of participation in neural transmission, should be considered. Although the final characterisation of these junctional complexes must await freeze-fracture analysis⁷, on the basis of the frequency of these morphological peculiarities, retrospective examination of other models of CNS demyelination⁸ will reveal whether or not these specialisations are features only of immune-mediated demyelination.

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Supersensitivity to the cyclic GMP response to glutamate during cerebellar maturation

THE action of certain neurotransmitters on receptors is associated with changes in the levels of cyclic nucleotides. Catecholamines, for example, increase cyclic AMP levels in both the central and peripheral nervous systems¹. In the adult brain the cerebellum contains the highest levels of cyclic GMP (ref. 2), and these can be altered *in vivo* by manipulation of excitatory pathways^{3,4} and intraventricular injection of γ -aminobutyric acid or glutamate^{3,5}, and *in vitro* by certain amino acid transmitter candidates^{6,7} and depolarising agents⁸. We show here that during a relatively short period in postnatal development, the excitatory amino acid, glutamate, elicits very large increases in the levels of cyclic GMP in slices of rat cerebellum, apparently located in the cells differentiating at this time.

Cerebella were rapidly excised from rats (Porton strain) of various ages, chopped (0.4 × 0.4 mm) at room temperature using a McIlwain chopper, and incubated at 37 °C in Krebs solution. The tissue was inactivated by boiling and, after homogenisation and centrifugation, cyclic nucleotides were estimated in the supernatant using commercial kits (Radiochemical Centre).

On addition of glutamate, cyclic GMP levels increased to reach a peak after 2–5 min irrespective of age (the time course at 8 d is shown in Fig. 1a). The extent to which they increased, however, was markedly age dependent. At 8 d (birth = day 1), maximal changes occurred at a glutamate concentration of 3 mM (Fig. 1b), with levels ($\pm$ s.e.m.) increasing from 0.56 ± 0.07 to 129 ± 5 pmol per mg protein, representing a >200-fold stimulation. This is considerably larger than any yet reported for non-tumorous nervous tissue. Changes of this magnitude were confined to slices from 8–14-d-old rats (Fig. 2). Either side of

Fig. 1 a, Time course and b, concentration dependence of the increase in cyclic GMP elicited by glutamate in slices of cerebellum from 8-d-old rats. Slices were preincubated (at about 1 mg protein ml⁻¹) for about 1.5 h (to allow cyclic nucleotide levels to stabilise) in Krebs solution containing (mM): NaCl (118), KCl (4.7), CaCl₂ (2.5), NaHCO₃ (25), KH₂PO₄ (1.18), MgSO₄ (1.19) and glucose (11), continuously gassed with a mixture of 95% O₂/5% CO₂ (pH 7.4) in flasks in a shaking water bath at 37 °C. At various times (a) or 5 min (b) after addition of glutamate, slices were removed using a Pasteur pipette, separated from the suspending medium by filtration and plunged into 1 ml of 50 mM Tris, 4 mM EDTA buffer, at 100 °C for 3–5 min. The interval between removal of slices and inactivation was <5 s. After homogenisation (through narrow gauge needles) and centrifugation, cyclic nucleotides in the supernatant fluid were measured. The protein content of the homogenate was estimated using the method of Lowry¹⁸. Points are the mean of 4–20 experiments, except for the 15-min point in a (mean of 2). Error bars are s.e.m. where this exceeds the dimensions of the symbol.

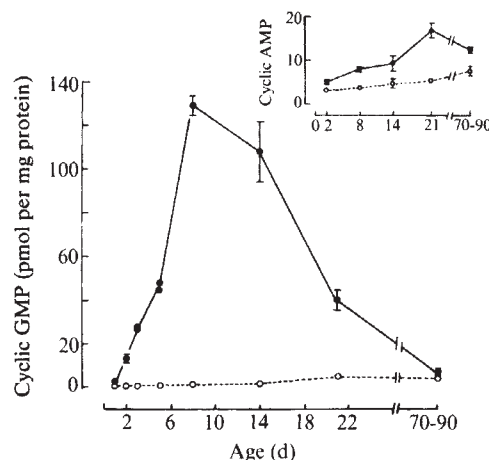
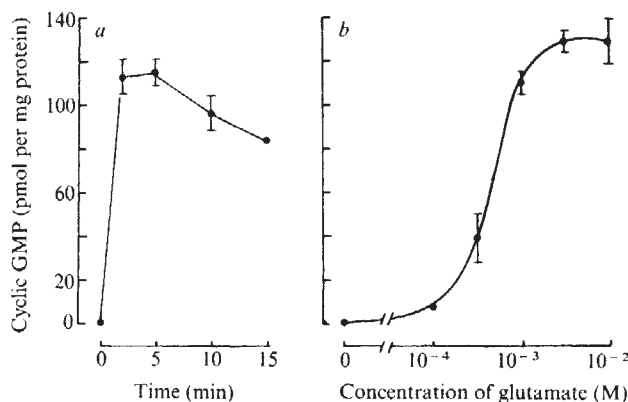


Fig. 2 Dependence on age. Solid circles show the cyclic GMP content in slices of cerebellum 5 min after the addition of glutamate (3 mM). Open circles are unstimulated levels, which show a developmental pattern similar to that found *in vivo* in mouse cerebellum², being fairly constant up to 8 d (at about 0.5 pmol per mg protein), increasing at 14 d (1.4 pmol per mg protein) and reaching adult values by 21 d (3–4 pmol per mg protein). At 1, 3 and 5 d, points show results of two experiments; at other ages, $n = 4$ –20. The insert shows the corresponding levels of cyclic AMP (pmol per mg protein) with respect to age. Error bars are s.e.m. where this exceeds the dimensions of the symbols.

this period, the degree of stimulation fell markedly, such that in the newborn it was sixfold, at 21 d tenfold, and in the adult (70–90 d) only twofold. Cyclic AMP levels also increased in response to glutamate, but in contrast, these were similar for all ages studied, and small, only about twofold (Fig. 2).

An important consideration in interpreting these results is the relative degree of preservation of the cells and their processes in the incubated slices with respect to age. The observed developmental pattern of the cyclic GMP response up to 21 d is unlikely to reflect variation in tissue damage, as electron microscopy of slices incubated for 1.5–2 h showed good preservation of morphology up to 21 d, but in slices from older rats, swelling and pyknosis in neuronal and glial structures became evident, suggesting that the response observed with adult slices may be an underestimate.

The question arises as to the possible localisation of the response. In the rat cerebellum, 97% of the cells are formed postnatally⁹. At 8 d, cell proliferation, including that of the precursors of the granule cells, the most abundant cell type in the adult cerebellum, is extensive. The following experiments were carried out in an attempt to narrow down cell types possibly responsible for the increase in cyclic GMP.

The dividing cells were eliminated *in vivo* using the mitotic inhibitor hydroxyurea (HU); injection of HU at 6 d (2 mg per g body weight) results in an almost complete loss of the external germinal layer by 8 d^{10,11}, corresponding to a 35% decrease in cell numbers compared with controls (in terms of DNA content¹¹). In slices from 8-d-old, HU-treated rats, however, both basal and maximal glutamate-stimulated cyclic GMP levels were increased by 100% and 80%, respectively, compared with slices from untreated rats (Table 1a), indicating a localisation predominantly in those cells undergoing differentiation as opposed to proliferation.

The intracerebellar nuclei were not a major site of the response, as the increase of cyclic GMP levels in slices from the ventral two-thirds of the cerebellum (where the nuclei are found) was similar to that in slices from the dorsal one-third (in which the nuclei would be excluded) and from whole cerebellum (Table 1b).

In addition, if the response resided in the mature granule cells (excitatory interneurons), it would be anticipated (unless the early-maturing granule cells are unusual) that the measured

Table 1 Effect of removal of proliferating cells and intracerebellar nuclei on basal and glutamate-stimulated levels of cyclic GMP

	<i>n</i>	Cyclic GMP (pmol per mg protein)	
		Basal	+ Glutamate (3 mM)
<i>a</i> Treatment			
Saline	4	1.41 ± 0.13	167 ± 8
Hydroxyurea	4	2.87 ± 0.52	306 ± 13
<i>b</i> Part of cerebellum			
Whole	2	1.24, 0.84	131, 136
Dorsal, one-third	2	1.35, 1.32	141, 142
Ventral, two-thirds	2	0.92, 0.66	115, 132

In *a*, rats were injected on day 6 either with hydroxyurea (2 mg per g body weight, intraperitoneally) to delete proliferating cells, or with an equivalent volume of saline; cerebella were used 2 d later. In *b*, either whole cerebella (8 d) were chopped, and incubated as usual, or the dorsal one-third was separated by hand using a razor blade, and chopped and incubated separately from the ventral two-thirds. (The intracerebellar nuclei are found in the latter part.) Data in *a* are given as mean ± s.e.m.; in *b* individual results are shown.

degree of stimulation would parallel the large increase in their numbers over 7–15 d after birth¹², contrary to our findings.

Consequently, much of the glutamate-induced increase in cyclic GMP in the immature cerebellum is likely to originate in the cells formed prenatally and/or in cells formed before the eighth day (Purkinje cells, Golgi cells, inhibitory interneurons, glial cells).

The period of maximal elevation of cyclic GMP levels by glutamate (8–14 d) coincides with extensive morphological, electrical and biochemical maturation of the cerebellum¹³; by 21 d, the tissue is essentially mature. Assuming the response to be localised in the neurones, the magnitude of the observed changes in cyclic GMP and their confinement to this period in development might have implications regarding a role for transmitter-induced changes in cyclic GMP in maturing neurones in the cerebellum, for example, in the stabilisation of synapses¹⁴. We would add that this apparent 'supersensitivity' to glutamate does not extend to other putative neurotransmitters with action supposedly linked to cyclic nucleotide-generating systems. Changes in cyclic AMP levels induced by noradrenaline at 8 d (maximally a two- to threefold increase, J.G. and R.B., in preparation) were similar to those reported for the adult^{15,16} and, in contrast to the action of acetylcholine in slices of adult rabbit cerebellum¹⁷, the cholinergic agonist, carbachol, at concentrations of up to 1 mM, produced no significant change in cyclic GMP levels in slices of rat cerebellum at 8 d (J.G. and R.B., in preparation).

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Calcium-dependent protein phosphorylation during secretion by exocytosis in the mast cell

IN many secretory cells materials destined for secretion are packaged in membrane-limited granules and are extruded by exocytosis¹. This process is known to require metabolic energy and calcium^{2–6}. Calcium probably acts as a common mediator in stimulus-secretion coupling², but the molecular mechanisms involved are unknown. Increasing evidence that many physiological processes are regulated by phosphorylation of proteins⁷ has led to the suggestion that calcium-dependent phosphorylation of proteins may be involved in secretory responses^{8–13}. We have sought evidence for this possibility in the mast cell, the rapid and extensive secretory activity of which conforms to the general pattern of exocytosis dependent on energy and calcium^{4,14–20}. Here we report a rapid phos-

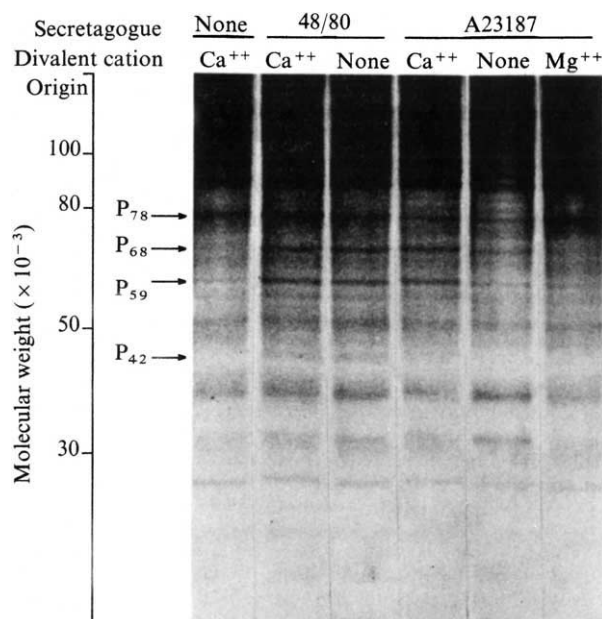
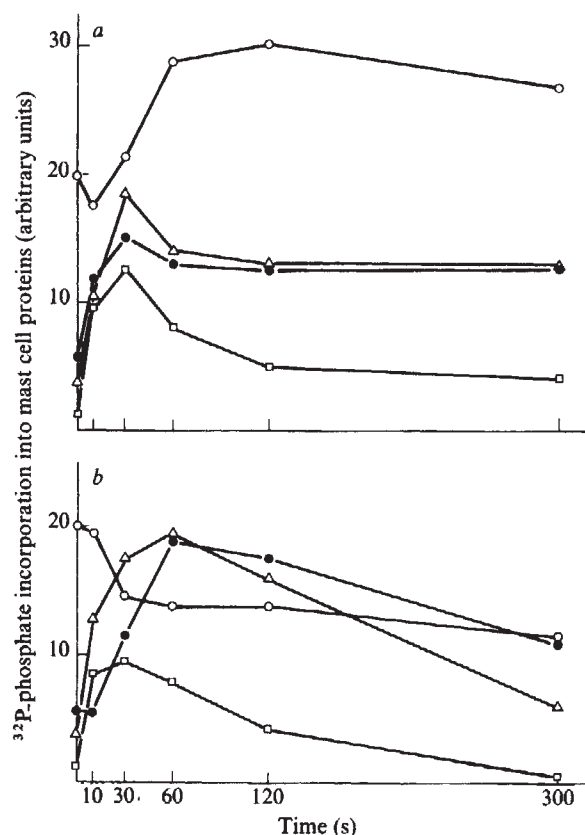


Fig. 1 Effect of 48/80 and A23187 on endogenous phosphorylation of mast cell proteins. Peritoneal mast cells derived from 15 Sprague-Dawley male rats (~400 g) were collected and purified as described previously²⁸. Mast cells (80–90% purity, 1.5×10^6 cells ml^{-1}) were preincubated in a standard buffer (HEPES 5 mM, pH 7.0, NaCl 150 mM, KCl 5 mM and glucose 5.5 mM) in the presence of CaCl_2 1 mM, 0.1% bovine serum albumin and 0.75 mCi ml^{-1} carrier-free ^{32}P -phosphate (NEN, 9120 Ci mmol^{-1}) for 1 h at 37°C. The cells were centrifuged at 180g for 5 min at 4°C and resuspended in 5 ml standard buffer in the presence of 0.1 mM EGTA. The cells were centrifuged twice more, each time followed by a resuspension in the standard buffer. Mast cell suspensions (0.2 ml) were introduced into plastic tubes containing 48/80 $1 \mu\text{g ml}^{-1}$, A23187 $1 \mu\text{g ml}^{-1}$, calcium 1 mM or magnesium 1 mM, in 0.1 ml standard buffer. Following 1 min incubation at 37°C, 150 μl of a stop solution (10% sodium dodecyl sulphate (SDS; w/v), Tris-HCl 100 mM, pH 7.4, β -mercaptoethanol 5 mM, sucrose 0.1 g ml^{-1} and bromophenol blue tracking dye 0.02 mg ml^{-1}) was added and the tubes were placed in a boiling water bath for 2 min. Electrophoresis on 10% polyacrylamide gels, protein staining of the gels, and autoradiography were carried out as described previously¹³. All radioactive bands disappeared after treatment of the samples with pronase but not with ribonuclease, using conditions described previously¹³, and thus represent phosphoproteins. The apparent molecular weights of the phosphorylated protein bands indicated by the arrows (78,000, P_{78} ; 68,000, P_{68} ; 59,000, P_{59} ; 42,000, P_{42}) were determined by calibrating the gel with standard proteins of known molecular weight. Degranulation, used as a measure of mast cell secretion, was monitored by light microscopy²².

phorylation of several proteins in rat peritoneal mast cells stimulated to secrete either by the classic mast cell secretagogue 48/80 (refs 15 and 21–23) or by the calcium ionophore A23187 (refs 22–25).

Purified rat peritoneal mast cells, which had been preincubated for 1 h with $^{32}\text{P}_i$, were incubated for 1 min with 48/80 or A23187 in the presence or absence of calcium or magnesium. The cells were then dissolved in sodium dodecyl sulphate and subjected to polyacrylamide gel electrophoresis (PAGE) and autoradiography. Addition of compound 48/80 $1\text{ }\mu\text{g ml}^{-1}$, which caused extensive degranulation of the mast cells within 1 min, resulted in an increased phosphorylation of four proteins (of apparent molecular weights 78,000, 68,000, 59,000 and 42,000) (Fig. 1, compare lanes 1 and 2). This change in phosphorylation elicited by 48/80 did not require the presence of extracellular calcium (Fig. 1, lane 3). In these experiments, mast cell secretion elicited by 48/80 also occurred in the absence of calcium, in agreement with evidence that this secretagogue acts largely through mobilisation of intracellular calcium^{22,23,25,26}. The general pattern of phosphorylation seen in response to 48/80 was highly reproducible (14 experiments), although the increase in radioactive phosphate incorporated into these four proteins varied from one experiment to another. The phosphorylation of several other proteins was sometimes influenced by 48/80 (and A23187), but this effect was not consistent.

Fig. 2 Phosphorylation of mast cell proteins as a function of incubation time with 48/80 (a) or A23187 (b). Mast cells (0.2 ml), isolated and purified as described in Fig. 1, were introduced into plastic tubes containing either 48/80 $1\text{ }\mu\text{g ml}^{-1}$ or A23187 $1\text{ }\mu\text{g ml}^{-1}$, in 0.1 ml standard buffer containing calcium 1 mM. At the times indicated, 150 μl of stop solution was added and the phosphorylation of mast cell proteins was analysed by SDS-PAGE and autoradiography¹³. The amount of ^{32}P -phosphate incorporated into four proteins, P_{78} (○), P_{68} (△), P_{59} (●) and P_{42} (□), was determined by scanning the autoradiographs with a Canalco G-II microdensitometer. The results are expressed in arbitrary units²⁹. Results qualitatively similar to those shown here were obtained in four other experiments.



Phosphorylation of the proteins with apparent molecular weights of 68,000, 59,000 and 42,000 was evident within 10 s after addition of 48/80, whereas phosphorylation of the 78,000 protein was not evident until 30–60 s after addition of the secretagogue (Fig. 2). After being maximally phosphorylated, these four proteins displayed different rates of dephosphorylation. The fact that compound 48/80 binds almost exclusively to receptors on mast cell membranes²⁷ indicates that the proteins phosphorylated in response to this secretagogue are derived from mast cells rather than from other contaminating peritoneal cells (10–20%).

As in the experiments with 48/80, mast cells incubated with A23187 $1\text{ }\mu\text{g ml}^{-1}$ in the presence of calcium 1 mM showed increased phosphorylation of the proteins with apparent molecular weights of 68,000, 59,000 and 42,000 (Fig. 1, compare lanes 1 and 4). When calcium was omitted from the incubation medium, A23187 had a diminished effect on the phosphorylation of these proteins (Fig. 1, lane 5). In the absence of calcium, mast cell secretion was also diminished, in agreement with studies demonstrating only a feeble stimulant effect of A23187 on secretion under similar conditions^{24,25}. The residual effect on mast cell secretion and on protein phosphorylation seen with A23187 in calcium-free medium was abolished by magnesium (Fig. 1, lane 6). Magnesium has been shown to reduce residual mast cell secretion in a low calcium medium²⁴. The phosphorylation of the 78,000 band was decreased by A23187 both in the presence and in the absence of calcium but not in the presence of magnesium (Fig. 1). The time course of phosphorylation and dephosphorylation of the 68,000, 59,000 and 42,000 proteins (but not that of the 78,000 protein) in response to A23187 and calcium was generally similar to that observed in the presence of 48/80 (Fig. 2).

These results demonstrate that both 48/80, which is thought to mobilise calcium from intracellular stores, and A23187, which promotes entry of calcium into the cell, stimulate the phosphorylation of the same proteins at a time when the secretory response is still developing. Calcium-dependent protein phosphorylation may therefore be involved in stimulus-secretion coupling. As yet, however, the evidence that the two phenomena are causally linked is only suggestive.

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Bromocriptine-induced inhibition of plasma dopamine, noradrenaline and adrenaline responses to LH-RF

IN addition to their regulatory role on pituitary hormones, hypothalamic-hypophyseal regulatory hormones may function in brain as neurotransmitters. Thus, in addition to the considerable evidence in laboratory animals suggesting that the release of luteinising hormone releasing factor (LH-RF) is regulated by brain amines¹, further evidence suggests that LH-RF may function at central nervous system synapses. It is found within synaptosomes and vesicle-like structures in various areas of brain²; it influences animal behaviour through a direct action in brain³, and microiontophoretic applications of LH-RF to neurones in the mediobasal hypothalamus and preoptic area are associated with prominent short-term changes in neuronal excitability, the most frequently observed effect being decreased excitability⁴. It has been suggested that axon collaterals within a putative peptidergic neuronal network may function in feedback circuits regulating activity of the tuberoinfundibular system and to inform other brain regions about the activity of this system. It seems reasonable to speculate that LH-RF release from such peptidergic neurones may act as a neurotransmitter or modulator to regulate the activity of certain central aminergic neurones. Thus, we examined the effects of LH-RF on plasma catecholamines in normal men, and found a rapid decrease in plasma concentrations of dopamine, noradrenaline and adrenaline (Fig. 1). Furthermore, bromocriptine, a dopamine receptor agonist, entirely prevented this depressant effect of LH-RF on plasma catecholamines.

In men kept supine for 2 h in the absence of bromocriptine, basal plasma concentrations of dopamine, noradrenaline and adrenaline were 58 ± 1 (mean $\pm$ s.e.), 273 ± 3 and 92 ± 4 pg ml⁻¹, respectively. These are similar to values we have reported elsewhere^{6,7}. LH-RF produced rapid decreases in plasma concentrations of all three catecholamines (Fig. 1). Plasma concentrations of dopamine, noradrenaline and adrenaline 5 min after LH-RF administration were 65 ± 3 , 77 ± 3 and 48 ± 2 % of the pre-LH-RF basal levels, and 20 min after LH-RF, decreased further to 34 ± 3 , 35 ± 1 and 24 ± 0.5 % of basal levels.

We have reported elsewhere that bromocriptine decreases plasma concentrations of dopamine, noradrenaline and adrenaline⁷. However, 4 h after bromocriptine administration, plasma concentrations of all three catecholamines had returned to basal levels. LH-RF administered at this time failed to suppress plasma concentrations of dopamine, noradrenaline and adrenaline (Fig. 1). Thus, bromocriptine interfered with the effect of LH-RF on plasma catecholamines at a time when its own effect on catecholamine release had apparently ended, although its effect on serum prolactin was still present.

Bromocriptine seems to produce many of its effects through an action on dopamine receptors⁸. Although evidence has been presented for an action on postsynaptic dopamine receptors⁹, more recent evidence supports an effect on presynaptic receptors¹⁰. We have suggested that bromocriptine may decrease plasma catecholamine concentrations by an action on presynaptic receptors of unknown central catecholaminergic neurones mediating sympathetic outflow, although a similar action on peripheral neurones could not be ruled out⁷.

It seems reasonable to speculate that LH-RF may act in a manner similar to that of bromocriptine. Alternatively, LH-RF

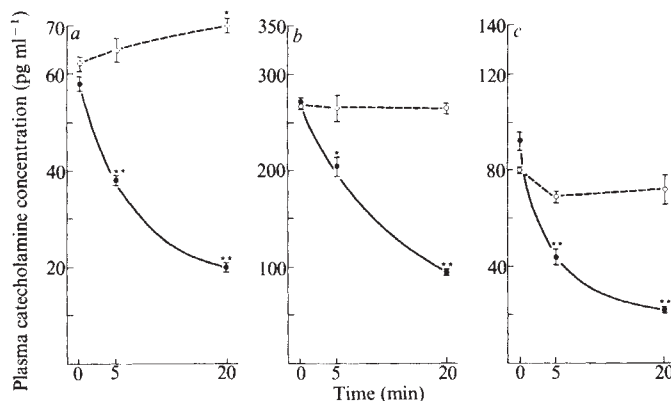


Fig. 1 Plasma dopamine, noradrenaline and adrenaline responses to LH-RF in the absence (●) and presence (○) of bromocriptine. Studies were carried out in three normal men, aged 36, 38 and 50 yr. An indwelling intravenous cannula was inserted into an ante-cubital vein of each subject on two occasions, with the subjects supine after fasting for 14 h. Blood (12 ml) was sampled for plasma catecholamines and serum prolactin, LH and FSH on each occasion, 1 and 5 h after cannula insertion and before administration of LH-RF. Subjects remained supine during this period except for a 5-min period of standing, 2 h after cannula insertion. LH-RF, 100 µg, was administered intravenously as a bolus and blood was sampled further, 5, 20, 60 and 120 min later. On the first occasion, bromocriptine had been taken orally 2.5 mg daily for 3 d, then 2.5 mg q12 h for another 4 d. A final dose of bromocriptine was administered 1 h after cannula insertion, that is, 4 h before LH-RF. On the second occasion, no bromocriptine had been taken for 2 weeks and none was administered during that part of the study. This allowed comparison of the plasma catecholamine response to LH-RF in the presence and absence of bromocriptine. Blood (2 ml) for catecholamine assay was transferred to tubes containing 4 mg of dithiothreitol and 20 µl of EGTA solution, 100 mg ml⁻¹. Plasma was separated and stored at -70°C until assay for catecholamines⁵. a, Dopamine; b, noradrenaline; c, adrenaline. * $P < 0.01$, significantly different from basal level. ** $P < 0.001$.

might act on presynaptic receptors of peripheral catecholaminergic neurones to decrease plasma catecholamines. It might act on dopamine receptors¹¹ or α -adrenoreceptors¹² to inhibit noradrenaline release from noradrenergic neurones as we discussed for bromocriptine⁷. It might also decrease dopamine release from peripheral dopaminergic or noradrenergic neurones. LH-RF-induced depression of adrenaline release, most of which probably comes from the adrenal medulla, is more difficult to explain by a peripheral rather than a central mechanism. If the LH-RF effect on plasma adrenaline is centrally mediated, it seems more probable that the effects on plasma dopamine and noradrenaline are also mediated centrally rather than peripherally.

Further support for the suggestion that LH-RF may decrease plasma catecholamines by acting directly on presynaptic receptors of catecholaminergic neurones is provided by our observations that bromocriptine prevents the LH-RF-induced depression of plasma catecholamines. Bromocriptine interfered with this effect of LH-RF at a time when its own effect on plasma catecholamines had ended. However, as the effect of bromocriptine on serum prolactin levels persisted at this time, it is possible that it still occupied dopamine receptors.

Further evidence that LH-RF may act on central catecholaminergic neurones is provided by our observations in rats that LH-RF decreased hypothalamic dopamine- β -hydroxylase activity (G.R.V.L. and R. Mascardo, unpublished).

In addition to its well known effects on serum LH and FSH, LH-RF-increased serum prolactin in our normal men (Table 1). Such an effect might be mediated directly on anterior pituitary

Table 1 Serum prolactin, LH and FSH responses to LH-RF in the absence and presence of bromocriptine

Time (min)	Prolactin (ng ml ⁻¹)*		LH (mU ml ⁻¹)*		FSH (mU ml ⁻¹)*	
	Control	Bromocriptine†	Control	Bromocriptine†	Control	Bromocriptine†
Pre-LH-RF‡	3.9 ± 0.2	<2.0	2.2 ± 0.2	2.3 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
LH-RF§						
+20	5.7 ± 0.6	<2.0	13.9 ± 0.9	13.6 ± 3.5	4.6 ± 0.4	3.1 ± 0.2
+60	5.2 ± 0.7	<2.0	10.4 ± 1.9	12.9 ± 2.6	3.6 ± 0.1	3.6 ± 0.7
+120	4.0 ± 0.3	<2.0	6.6 ± 1.1	7.2 ± 1.3	3.2 ± 1.0	3.7 ± 0.4

* Serum prolactin, LH and FSH were assayed by standard radioimmunoassay methods described elsewhere. All values are concentration means ± s.e.m.

† Bromocriptine 2.5 mg administered orally 240 min before LH-RF.

‡ All Pre-LH-RF values are pooled means of 18 determinations, six samples taken at intervals during the 4 h before LH-RF administration from each of the three subjects.

§ LH-RF, 100 µg, was administered as an intravenous bolus.

|| Significantly different from Pre-LH-RF concentration at $P < 0.05$ using analysis of variance and Duncan's multiple range test.

cells or indirectly in the median eminence. If LH-RF inhibits dopamine release from tuberoinfundibular dopaminergic neurones, as speculated above for catecholamine neurones generally, and dopamine acts to inhibit prolactin secretion, then increased prolactin secretion could be expected following LH-RF administration. Bromocriptine prevented this LH-RF-induced increase in serum prolactin, providing another parallel with the interference of LH-RF-induced effects on plasma catecholamines. There were no apparent effects of bromocriptine on the plasma LH and FSH responses to LH-RF.

If the effects of LH-RF on plasma catecholamine concentrations are mediated through presynaptic receptors as suggested above then the presynaptic receptor mechanisms involved must be functionally intact to produce a normal response. Thus, the plasma catecholamine response to LH-RF may provide an index of normal function of certain catecholaminergic neurones in man. As a corollary, dysfunction of certain catecholamine neurones might be demonstrated by abnormal plasma catecholamine responses to LH-RF. Support for this hypothesis is provided by the demonstration that in contrast to normal men, patients with acromegaly show no changes in plasma dopamine, noradrenaline or adrenaline following LH-RF administration¹³. Thus, acromegaly may represent a disorder of catecholaminergic neurones. Furthermore, the plasma catecholamine response to LH-RF may prove useful as a test of catecholaminergic dysfunction in a wide variety of disorders such as Parkinson's disease, essential hypertension, autonomic neuropathies and poorly defined hypothalamic disorders.

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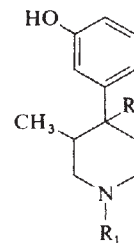
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New structural concepts for narcotic antagonists defined in a 4-phenylpiperidine series

NARCOTIC antagonists are generally considered as *N*-methylcyclopropyl or *N*-allyl (for example, nalorphine) type derivatives of *N*-methyl (for example, morphine) agonists. This type of substitution has often been considered necessary for potent narcotic antagonist activity^{1,2}. However, in the 4-phenylpiperidine series (as in meperidine) where a methyl substitution on the nitrogen has been associated with agonist activity, the substitution of an *N*-allyl derivative has not produced narcotic antagonists³⁻⁵. There are compounds, such as L-metazocine^{6,7} and profadol⁸, which contain a methyl-substituted basic nitrogen, and possess narcotic antagonist properties, but these are also agonists and their antagonist activity is relatively weak. During the pharmacological investigation of a new series of phenylpiperidines we discovered an *N*-methyl derivative that was a pure narcotic antagonist devoid of agonist effects. We also observed that nitrogen substituents that had increased agonist potency in the meperidine series⁹ when substituted in compounds of the present series surprisingly augmented antagonist potency to the level observed with naloxone⁴. Examination of the inhibition of specific ³H-naloxone binding of these derivatives in bovine striatal membranes demonstrated that their relative potency was due to differences in receptor interaction. We used the most potent member of the series to study its stereoselectivity. We report here the structure-activity relationships of some 1,3,4-trialkyl-4-phenylpiperidines that clearly demonstrate the importance of a 3-methyl substituent in contributing narcotic antagonist properties within the phenylpiperidine series as a whole. These compounds may well represent the first new class of pure narcotic antagonists since the discovery of naloxone.



Narcotic antagonist measurements for the 1,3,4-trialkyl-4-phenylpiperidine series are shown in Table 1. The AD₅₀ (dose that reduced the morphine response by 50%) for the *N*-methyl compound (I) was 0.24 mg per kg in rats and 1.0 mg per kg in mice. These values are comparable to the AD₅₀ values for

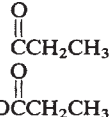
nalorphine of 0.4 mg per kg and 0.45 mg per kg in rats and mice, respectively. The allyl (II) and methylcyclopropyl (III) substitution did not increase the antagonist potency, as might have been expected in a multicyclic series (for example, morphinan⁴ and benzomorphan¹⁰). There was an increase in antagonist potency when the *N*-methyl was changed to *N*- β -phenethyl (IV) or *N*-2-propiofenone (V). Antagonist potency of the *N*-propiofenone derivative (V) was comparable to that of naloxone. Resolution of the 2-propiofenone (V) into its (+)-isomer (VII) and (–)-isomer (VI) provided only partial separation of activity, as both were antagonists, with the (+)-isomer being 2–6 times more potent than the (–)-isomer.

The 1,3,4-trialkyl-4-phenylpiperidines (Table 1) were pure antagonists as judged by the following observations. They were without measurable narcotic agonist activity (analgesia) at 100 mg per kg, given subcutaneously (s.c.), in the mouse writhing analgesic test, where compounds with mixed agonist/antagonist activity (such as nalorphine) exhibit analgesic effects (method previously described¹¹). More convincingly, in an *in vitro* measurement sensitive to narcotic agonist effects and free of the influence of metabolism, the *N*-propiofenone (V) was without measurable narcotic agonist effects. This lack of effect *in vitro* was on the twitch height of the electrically stimulated longitudinal muscle of the guinea pig ileum (method previously described¹¹). Compounds with mixed agonist/antagonist properties depress the twitch height in relation to the potency of their agonist effects, whereas pure antagonists (such as naloxone) show negligible depressant effects¹⁰. Compound V, like naloxone, lacked agonist activity at 10 μ g ml^{–1}, but completely antagonised the narcotic agonist effects of morphine at a concentration of 10 ng ml^{–1}.

The increase in antagonist potency observed when the *N*-methyl is changed to *N*-propiofenone is particularly interesting because this substitution is known to enhance agonist activity in the meperidine and prodine series⁹. Table 2 shows the receptor affinities of these 1-alkyl- β -3,4-dimethyl-4-phenylpiperidines as measured by their inhibition of ³H-naloxone binding to bovine striatal membrane homogenates in the presence and absence of NaCl. The rank order of the IC₅₀ values for compounds I–V closely parallels the observed *in vivo* antagonist potencies given in Table 1. The *N*-methyl (I), *N*-allyl (II) and *N*-methylcyclopropyl (III) derivatives all had relatively high IC₅₀ values. However, substitution of the *N*-methyl group by either the *N*- β -phenethyl (IV) or *N*-2-propiofenone (V) groups resulted in a 100-fold increase in competitive binding, comparable to that of nonradioactive naloxone. The ³H-naloxone binding of these derivatives was essentially unchanged in the presence of NaCl, confirming their binding to be that of a narcotic antagonist¹².

The importance of the methyl at position 3 on the piperidine ring is shown in Table 1. Compound IX, a reported narcotic agonist¹³ lacking the 3-methyl moiety, is devoid of narcotic antagonist activity. Table 1 also shows the structural specificity of the antagonist activity in that the 3 α -methyl isomer (VIII) (*cis*-3-methyl, 4-methyl) is about 50 times less active than the 3 β -methyl isomer (I) (*trans*-3-methyl, 4-methyl). The significance of the 3-methyl substitution is further indicated in Table 1. A methyl substituted on the position of the ketobemidone molecule provided a weak antagonist (X), which contrasts sharply with the potent agonist effects of ketobemidone¹⁴. The 3-methyl ketobemidone derivative (X) is a mixed agonist/antagonist producing inhibition of writhing (analgesia)

Table 1 1,3,4-trialkyl-4-phenylpiperidines and their narcotic antagonist activities

R ₁	R ₂	Configuration	Antagonist activity AD ₅₀ (mg per kg, s.c.)	
			Rats	Mice
I CH ₃	CH ₃	β^*	0.24	1.0
II CH ₂ CH=CH ₂	CH ₃	β	0.47	0.98
III 	CH ₃	β	0.72	0.72
IV CH ₂ CH ₂ Ph	CH ₃	β	0.11	0.14
V CH ₂ CH ₂ CPh ($\pm$ isomer)	CH ₃	β	0.056	0.049
VI CH ₂ CH ₂ CPh (– isomer)	CH ₃	β	0.050	0.14
VII CH ₂ CH ₂ CPh (+ isomer)	CH ₃	β	0.023	0.025
VIII CH ₃	CH ₃	$\alpha^\dagger$	33	39
IX CH ₃	CH ₃	– $\ddagger$	None	None
X CH ₃		β	21	38
XI CH ₃		β	6.2	9.9
Pentazocine			20	14
Nalorphine			0.40	0.45
Naloxone			0.022	0.079

Compounds I–VIII were prepared in a multiple-step procedure starting from 3-methoxyphenylacetone. The isomeric configurations were confirmed by X-ray crystallography and ¹³C-NMR analysis. Compounds IX (ref. 13), X (ref. 16) and XI (ref. 17) were synthesised by modified published procedures. Water-soluble salts were prepared in all instances. The ability of each test compound to reduce the opiate effect of morphine was measured in mice by determining the antagonism of the morphine-induced Straub tail and increased locomotion. Fasted male Cox Standard albino mice (17–21 g) were used. Morphine sulphate was administered at 40 mg per kg, s.c., and the presence or absence of the Straub tail and increased locomotion were noted and scored 15 min later. The test antagonist was administered s.c. at various doses 15 min before the morphine sulphate. The ability of the test compound to reduce or antagonise the analgesic effect of morphine was measured in the rat tail heat analgesic test (previously described¹⁰). The only change in the method was to increase the stimulus by using 7 A currents in the heating wire. We routinely measured the analgesic effect of morphine sulphate 10 min after the s.c. administration of 10 mg per kg. The elevations in reaction time following morphine were 2.5–8.6 s, with the average of 24 groups being 4.8 s. The test antagonist was administered s.c. at various doses 20 min before the morphine sulphate. The increase above controls for the morphine group was compared to the group receiving both morphine and the antagonist, and the % reduction in the morphine response determined. The AD₅₀ value, determined by “the use of the regression line in reverse”¹⁸ was the dose required for a 50% reduction in the response to morphine.

* *trans*-3-methyl, 4-R.

† *cis*-3-methyl, 4-R.

‡ Compound IX lacks the 3-methyl group.

Table 2 Inhibition of ^3H -naloxone binding by 1-alkyl- β -3,4-dimethyl-4-phenylpiperidines in the presence and absence of NaCl

Compound	Concentration of compound at 50% inhibition (IC_{50} , nM)		Na shift (+NaCl/-NaCl)
	+NaCl	-NaCl	
I	50	100	0.5
II	60	90	0.7
III	60	60	1
IV	0.4	1.5	0.3
V	2	1	2
Naloxone	1.2	1.6	0.8
Levorphanol	60	2	30

Bovine striatal membranes of 0.6 mg protein were incubated at 24 °C for 15 min in 1 ml of 100 mM Tris-Cl, pH 7.4 buffer containing 2 nM ^3H -naloxone (38 Ci mol $^{-1}$, NEN) and 100 mM NaCl as indicated. Samples were centrifuged at 1,085g for 10 min. Radioactivity in the pellet was determined by a liquid scintillation method. The IC_{50} value was obtained by plotting % inhibition against a 4-decade change in concentration of each compound in a semilog plot. Specific binding of ^3H -naloxone was defined as the difference in binding between samples with and without 1 μM naltrexone. In control samples, ^3H -naloxone was specifically bound to bovine striatal membranes at 40.8 ± 1.5 and 119.7 ± 2.6 fmol per mg protein in the absence and presence of 100 mM NaCl, respectively. Other experimental conditions were followed according to published methods¹⁹.

in mice at 10 mg per kg, s.c. Hydroxylation of the phenyl in β -prodine, a compound already possessing the appropriate 3-methyl, changed this potent agonist¹⁵ into a narcotic antagonist (XI) with a greater potency than that of pentazocine, but lacking measurable agonist activity.

In summary, we report here the discovery of several novel phenylpiperidines which are potent narcotic antagonists without measurable agonist properties. We believe that this defines a new class of narcotic antagonists in which the 3 position of the piperidine ring rather than substitution at the nitrogen is the area critical for determining antagonist activity.

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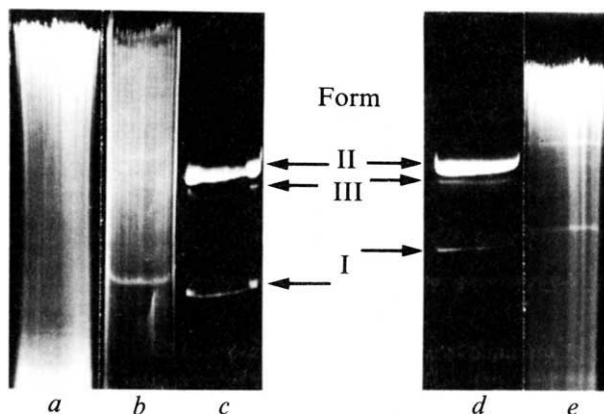
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Viral sequences related to a human skin papillomavirus in genital warts

GENITAL WARTS (condylomata acuminata) are benign tumours which are mostly venereally transmitted¹; their malignant conversion has been observed only rarely (see refs 2, 3 for reviews). Typical viral particles, long believed to be those of the human papillomavirus (HPV) found in skin warts⁴, were observed irregularly in these lesions⁵⁻⁷. Epidemiological¹ and serological⁶ data, as well as molecular hybridisation experiments showing the absence of DNA sequences homologous to skin HPV DNA in condylomas^{8,9}, have raised the possibility that these lesions are due to a distinct HPV. However, the demonstration of at least four distinct HPV types in skin lesions¹⁰⁻¹³ requires a reexamination of this problem. We have identified four types of HPV showing little, if any, DNA sequence homology and distinct serological properties: HPV type 1 (HPV-1) associated with deep plantar warts¹², HPV type 2 (HPV-2) isolated from common warts¹², HPV type 3 (HPV-3) found in flat warts and in flat wart-like lesions of patients suffering from epidermodysplasia verruciformis (EV)¹³ and HPV type 4 (HPV-4) isolated from morphologically distinct EV lesions (reddish or pityriasis versicolor-like plaques)¹³. HPV-4 is found more frequently than HPV-3 in patients with EV showing a malignant conversion of some lesions^{13,22}. We report here RNA-DNA hybridisation data indicating that genital warts are associated with an HPV showing little, if any, DNA sequence homology with HPV-1, HPV-2 and HPV-3 and a low extent of homology with an HPV-4 isolate taken as prototype in previous studies¹³.

Fig. 1 Agarose gel electrophoresis of DNAs extracted from genital warts. Warts obtained from seven patients (Table 1) were processed according to a modified Hirt method^{12,14}. Minced tissues (0.3-1 g) were incubated in 10 ml of 0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA, 0.5% SDS containing 50 $\mu\text{g ml}^{-1}$ of proteinase K (Merck). After a 6 to 10 h incubation at 37 °C, NaCl concentration was adjusted to 1 M, and the mixtures were kept at 4 °C overnight. Supernatants were extracted twice with phenol and DNAs were ethanol-precipitated and redissolved in 0.5-1 ml of 0.01 M Tris, 0.001 M EDTA, pH 7.9. Yields of DNA ranged from 0.04 to 1 mg as determined by absorbance at 260 nm. DNAs (30-300 μg per slot) were fractionated by electrophoresis in vertical 0.8% agarose slab gels (0.04 M Tris, 0.02 M ammonium acetate, 0.002 M EDTA, pH 7.8) for 20 h at 30 mA, taking HPV-1 form I, II and III DNA as standards (10 μg DNA per slot). Gels were stained in 5 $\mu\text{g ml}^{-1}$ ethidium bromide and electropherograms visualised under UV light, as shown for the DNAs extracted from genital warts of patients N.M. (a), W.K. (b) and M.B. (c) and for HPV-1 form I, II and III DNA (c, d). The slower migration of the genital wart HPV DNA is probably due to the high viscosity of the samples.



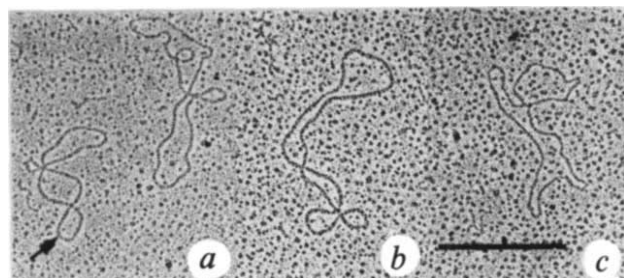


Fig. 2 Electron micrographs of circular HPV DNA molecules obtained from genital warts. DNAs were selectively extracted from lesions of patients W.K. (a), W.L. (b) and M.B. (c) and further purified by Agarose gel electrophoresis as described in the legend to Fig. 1. Bands with a mobility comparable to that of HPV-1 form I DNA molecules were sliced from the gel and DNA was eluted electrophoretically, mounted on collodion-coated grids, Pt-Pd shadowed and observed with a Siemens Elmiskop 101 electron microscope¹². The arrow indicates a molecule of replicative form of ΦX174 phage DNA (ΦX174 RF DNA), taken as length standard. The bar represents 0.5 μm.

The present study was carried out with genital warts obtained from seven patients (Table 1). Because the low virus content of lesions^{5,7,9} precluded virus purification, viral DNA was extracted selectively from the tissues^{12,14} and purified by Agarose gel electrophoresis¹⁵ (Fig. 1). Four of the DNA preparations did not yield detectable bands (Fig. 1a). However, preparations obtained from patients W.K., W.L. and M.B. showed bands migrating slightly more slowly than those of HPV-1 form I, II or III DNA (Fig. 1b–e). Circular DNA molecules of about 2.5 μm, typical of HPV DNAs¹⁶ (Fig. 2), were observed in electrophoretic eluates of the bands corresponding to form I DNA. This result is in contrast to the previously reported failure to demonstrate the presence of a distinct papova-like circular DNA in condylomata acuminata⁹. Comparable length distributions were found for W.K. and M.B. HPV DNAs purified by Agarose gel electrophoresis (Fig. 3a, c) and for W.L. HPV

DNA molecules found in total DNA (Hirt supernatant) (Fig. 3b). The lengths of W.K., M.B. and W.L. HPV DNAs were 1.493 ± 0.08 , 1.439 ± 0.07 and 1.462 ± 0.08 ΦX174 RF DNA units, corresponding to 8,025, 7,735 and 7,860 base pairs, respectively. Molecular weights inferred from these values (5.2 – 5.3×10^6) are close to those determined for the DNAs of the HPVs so far identified^{12,13}. A second population of circular DNA molecules, about 5.1 μm long, was observed in the total W.L. DNA preparation (Fig. 3b). This could represent either HPV DNA dimers or, more probably, mitochondrial DNA molecules^{9,18}.

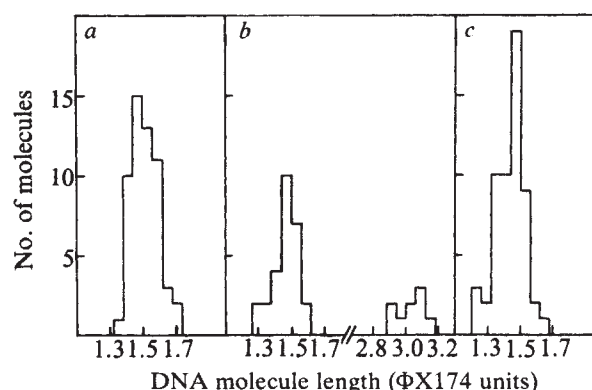


Fig. 3 Histograms of the lengths of genital wart HPV DNA molecules. DNA selectively extracted from lesions (b, W.L.) and further purified by Agarose gel electrophoresis (a, W.K.; c, M.B.) (see legend to Fig. 2) were mixed with ΦX174 RF DNA molecules. Contour lengths of relaxed circular molecules were measured on electron micrographs and expressed as fractional length of ΦX174 RF DNA¹².

Total DNA from genital warts, as well as purified form I DNA preparations (W.K. and M.B. HPV DNAs), were hybridised with RNAs complementary (cRNAs) to HPV-1, HPV-2, HPV-3 or HPV-4 DNAs^{12,13}. In these conditions^{12,13,20}, 50–60% of the radioactivity bound to the filters after hybridisation of

Table 1 Search for DNA sequences homologous to skin HPV DNAs in DNA extracted from genital warts

Patients	Location of lesions	DNA on filters	Radioactivity bound on filters (%) after hybridisation with labelled RNAs complementary to the DNAs of			
			HPV-1	HPV-2	HPV-3	HPV-4
W.K. (M, 28)	Meatus	Total	0.1	0.5	0.4	7.3
		Eluate	–0.3	0.1	–0.3	8.8
M.B. (M, 26)	Glans, prepuce	Total	0.1	–0.3	–0.4	4.2
		Eluate	0.3	0.2	0.1	5.1
W.L. (M, 26)	Prepuce	Total	0.1	0.9	1.1	4.9
N.M. (M, 44)	Meatus, prepuce	Total	0.3	0.1	1.1	3.6
W.B. (M, 64)	Prepuce	Total	0.2	1.0	0.8	5.5
B.B. (M, 30)	Groin, anus	Total	0.5	0.4	0.5	3.5
M.K. (F, 24)	Vulva	Total	0.5	0.3	0.3	5.0
		HPV-1	55.9	0.3	0.1	0.3
		HPV-2	0.2	58.3	0.5	0.1
		HPV-3	0.7	0.3	49.2	0.2
		HPV-4	0.5	–0.2	–0.1	52.5
		Thymus	0.1	0.3	0.1	0.8
		Liver	0.2	0.1	1.0	0.4

RNA–DNA filter hybridisation under paraffin was carried out as described by Kourilsky *et al.*²⁰ with tritiated RNAs (specific activity, 3 – 4×10^7 c.p.m. μg^{–1}) complementary (cRNAs) to the four HPV DNAs transcribed *in vitro* as previously described¹². HPV DNAs were obtained from a single plantar wart (HPV-1)¹², pooled hand common warts of a single patient (HPV-2)¹², and pooled scrapings of lesions of two patients with E.V. (HPV-3, HPV-4)¹³; these HPV-2, HPV-3 and HPV-4 preparations were those taken as prototypes in previous studies^{12,13}. Genital wart DNAs were selectively extracted from the lesions of seven patients according to a modified Hirt procedure^{12,14} (total DNA) or further purified by Agarose gel electrophoresis (eluate), as described in the legends to Figs 1 and 2. Nitrocellulose filters (two per assay) carrying heat-denatured prototypical HPV DNAs, W.K. and M.B. HPV DNAs (about 50 ng, as determined with ΦX174 RF DNA as a standard²¹), genital wart DNAs (2.5 – 7.5 μg), human fetal thymus or liver DNA (7.5 μg), as well as blank filters, were added with 4 μl of cRNA ($2,000$ c.p.m. per filter) in 0.30 M NaCl, 0.030 M sodium citrate – 50% formamide. Heat-denatured fetal human thymus DNA was added to the hybridisation mixture (0.5 μg per assay) to eliminate any hybridisation to cellular DNA on filters. Paraffin was added and filters were incubated for 15 h at 37°C , processed and counted as previously described¹². Sex and age (yr) of patients are given in parentheses.

homologous viral DNAs and cRNAs, whereas no radioactivity was retained using heterologous prototypical cRNAs and DNAs (Table 1). No significant hybridisation was obtained with any of the genital wart DNAs (total or form I) and HPV-1, HPV-2 or HPV-3 cRNAs. However, all the total DNA preparations, as well as purified form I DNA from W.K. and M.B. tumours, showed significant levels (3.5–8.8%) of hybridisation with the HPV-4 cRNA probe (Table 1).

Our results indicate that three known types of human papillomavirus, HPV-1, HPV-2 and HPV-3, are not generally associated with genital warts (see, however, ref. 22). On the other hand, we find that viral sequences related to a fourth type, HPV-4, are present in DNA of the tumours studied here. The present data do not, however, show to what extent the sequences are related, and this remains a subject for further study.

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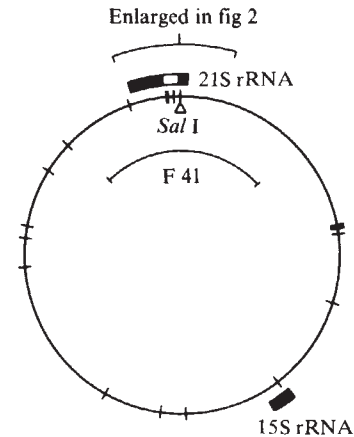


Fig. 1 The physical map of mtDNA from *S. cerevisiae* strain JS1-3D, showing the position of the rRNA genes and the recognition sites for endonucleases *Hind*II and *Hind*III and the single site for endonuclease *Sal*I (from refs 5, 10). The contour length is 72,000 base pairs. The region of the map enlarged in Fig. 2 and the segment of mtDNA retained in petite mutant F41 are indicated.

yeast mitochondrial ribosomes⁵, but, nevertheless, this RNA hybridises to non-adjacent fragments on the physical map of mtDNA⁶. Although this suggested that the gene for this RNA is split, like some eukaryotic nuclear genes⁷, the presence of partial gene duplications complicated the interpretation of our hybridisation experiments⁶. By electron microscopy of RNA–DNA hybrids we now present direct evidence for an insertion in this rRNA gene.

Figure 1 shows a simplified map of yeast mtDNA, and Fig. 2 gives a more detailed map of the region of the large rRNA cistron. The rRNA hybridises to the *Hind*II and *Hind*III fragments TD9, DT14 and TT1 (hatched in Fig. 2), but not to TD15 and the small 70-base pair fragment next to it⁶. Additional hybridisation experiments (unpublished) have shown that this is not due merely to partial gene duplication, because no homology was found between sequences hybridising with 21S rRNA in fragment TT1 and fragment TD9.

To prove that the rRNA gene is indeed split, hybrids were made between rRNA and the *Hae*III fragment indicated in Fig. 2. The rRNA was obtained by fractionating total mtRNA from *Saccharomyces cerevisiae* strain JS1-3D by agarose gel electrophoresis and eluting the 21S rRNA band 8. The *Hae*III fragment was isolated⁹ from the mtDNA of petite mutant F41. This petite has been derived from *S. cerevisiae* strain JS1-3D; it contains the segment of wild-type mtDNA indicated in Fig. 1 which has been shown by restriction enzyme digestion and hybridisation analysis^{6,10} to be identical to the corresponding wild-type sequence. Hybrids were formed in high formamide concentration to avoid DNA renaturation¹¹, and spread in conditions which allow a clear distinction between single-stranded RNA (kinky) and DNA (smooth) strands. Figure 3 shows two of the predominant hybrid molecules present in electron micrographs of these hybrids and the insert gives our interpretation of these molecules. The single-stranded DNA loop between two duplex hybrid sections proves that the mature rRNA is complementary to non-adjacent segments of mtDNA. Not a single full-size hybrid molecule without this loop was observed. The left–right orientation of the hybrid molecules relative to the map in Fig. 2 is determined by the morphology of the single-stranded ends, which shows that the left-hand end is RNA and the right-hand end DNA. This orientation was confirmed by the presence of rare hybrid molecules that had reannealed with a broken complementary DNA strand of the *Hae*III fragment, resulting in hybrids with a partially duplex end. This duplex end was present at the side of the short hybrid segment (segment c in Fig. 3).

An insert in the single gene for the large ribosomal RNA in yeast mitochondrial DNA

MITOCHONDRIAL DNA (mtDNA) contains genes for the ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) used in mitochondrial protein synthesis and for a limited number of proteins of the mitochondrial inner membrane^{1–4}. By a combination of genetic and physical mapping, the location of most of these genes on yeast mtDNA has been determined^{3,4}. There is a single gene for the RNA from the large subunit of

Table 1 Size of hybrid and single-stranded DNA regions in 42 rRNA hybrid molecules

Segment (see Fig. 3)	$\mu\text{m} \pm \text{s.d.}$	Size Base pairs
a	0.33 ± 0.02	830
c	0.20 ± 0.01	510
b	0.50 ± 0.03	1,160

Measurements of hybrid sections were calibrated with the circular replicative form of phage ΦX174 (5,375 base pairs²⁰), co-spread and measured on the same grid. Single-stranded DNA segments were calibrated using the single-stranded *HaeIII* fragment (4,300 nucleotides) present on the same grid.

The position of the rRNA gene on the map in Fig. 2 follows from the quantitative information on 42 hybrid molecules summarised in Table 1 and from the sections of the DNA previously shown to hybridise with rRNA^{6,10}. The location of the insert is determined by the size of section a (830 base pairs) and the position of the left-hand end of the *HaeIII* fragment¹², which we have mapped by double-digestion analysis. The size of the insert, determined from the size of loop b, is 1,160 base pairs. From the size of sections a–c, we infer that the rRNA cistron runs about 400–500 base pairs into fragment TT1.

Although inserts have previously been found in the nuclear rRNA genes of *Drosophila*^{11,13–16}, it has not yet been possible to decide if these split genes yield mature rRNA. With yeast mtDNA this is certain, because no other gene is present.

The region of the 21S rRNA on yeast mtDNA contains interesting structural and genetic elements, as indicated in Fig. 2. First, there is the 1,050-base pair insertion VI of Sanders *et al.*⁵ found by us^{5,6} and by Jacq *et al.*¹² in the ω^+ strains and lacking in the ω^- strains characterised by Slonimski *et al.*¹⁷. This 1,050-base pair insertion coincides with the intervening sequence in the rRNA gene within the limits of error of our measurements. Other interesting genetic loci are *rib-1* (chloramphenicol resistance) and *rib-2* (erythromycin resistance). We have argued previously¹⁸ that mutations at these loci affect the mitochondrial ribosome by altering the nucleotide



Fig. 2 The physical map of the segment of yeast mtDNA that contains the gene for the large 21S rRNA. The position of the cleavage sites for endonucleases *HindII*, *HindIII*, *HapII* and *SalI* on the mtDNA of *S. cerevisiae* strain JS1-3D are taken from refs 5, 6 and 10. Hatched fragments hybridise with 21S rRNA^{6,10}. The position on the map of the *HaeIII* fragment, used for electron microscopy of DNA–RNA hybrids, was determined by double-digestion analysis with endonucleases *SalI*, *HindII* and *HindIII* (unpublished). The position of the rRNA gene was deduced from the hybridisation results^{6,10} and the measurements in Table 1; from the size of the rRNA (3,700 nucleotides²¹) we infer that the left border of the rRNA gene must be close to the left *HapII* site in TD9, as drawn, but this has not been verified. The positions of the genetic loci are taken from ref. 10 and unpublished results; ω is a locus which affects the recombination of markers around it; there is a one-to-one correlation^{5,12} between the ω^+ allele and insertion VI of Sanders *et al.*⁵. Mutations at *rib-1* and *rib-2* confer resistance of mitochondrial protein synthesis to chloramphenicol and erythromycin, respectively. *rib-1* and *rib-2* are most probably located in the region indicated by the continuous line, but a position more to the right, indicated by the broken line, is not excluded.

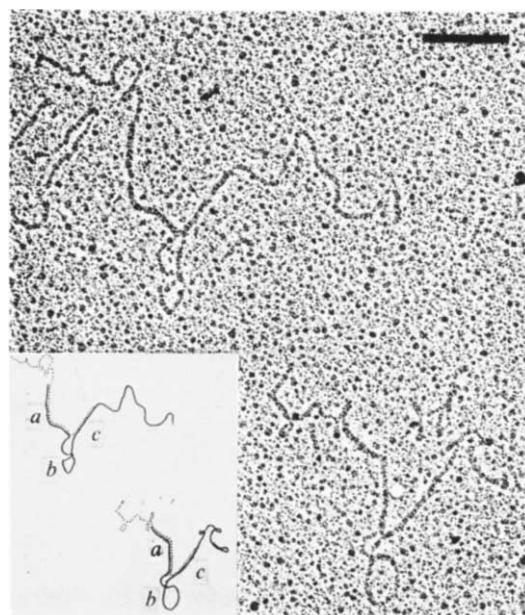


Fig. 3 Electron micrograph of RNA–DNA hybrids made between 21S rRNA and the denatured *HaeIII* fragment indicated in Fig. 2. The hybridisation and visualisation of hybrids was done by the procedure of Wellauer and Dawid¹¹ as described²², with the modification that the hybridisation was carried out for 2 h at 40 °C. Main figure electron micrograph showing two adjacent hybrid molecules; scale bar, 0.2 μm . Inset, interpretation of main figure; dotted lines are RNA, straight lines DNA.

sequence of rRNA. The precise mapping of these loci, on the one hand, and of the rRNA, on the other hand, now lends support to this suggestion.

In previous papers^{4,6,19} from our laboratory, it was suggested that the genes for subunit I of cytochrome *c*-oxidase (*oxi-3* locus) and for cytochrome *b* (*cob* locus) are split to account for the discrepancy between apparent locus size and protein product. Our present results re-emphasise this suggestion. Yeast mitochondria with their simple and well-characterised genome⁴ may well turn out to be an attractive experimental system for the study of the nature and evolution of gene inserts and the splicing enzymes required to process the primary transcripts of such genes.

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Yeast has a true stringent response

In bacteria there is a coordinate regulation of the synthesis of ribosomal RNA and ribosomal protein¹. This is most evident from studies of the rates of synthesis of ribosomal components as a function of the growth rate of the cell^{2,3}. Another example is the 'stringent response', in which cells deprived of an essential amino acid shut down the transcription of ribosomal RNA as well as that of messenger RNA for ribosomal proteins^{4,5}, but increase the synthesis of mRNA for certain biosynthetic proteins⁶. Although this stringent response is at least partially due

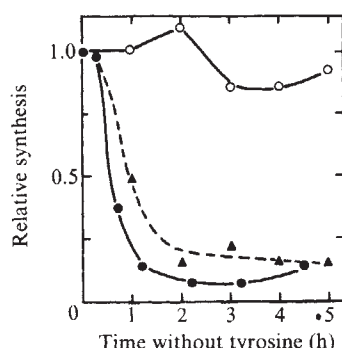


Fig. 1 Macromolecular synthesis in cells deprived of tyrosine. Protein synthesis (●): *Saccharomyces cerevisiae*, strain A364A, a, *ade*⁻, *ura*⁻, *his*⁻, *lys*⁻, *try*⁻, were cultured in synthetic complete medium at 23 °C as described elsewhere⁷, to a concentration of 1×10^7 . The cells were collected by filtration, washed and suspended in medium lacking tyrosine. At various times 5 ml of culture was added to a flask containing 2.5 μ Ci of ¹⁴C leucine (120 μ Ci μ mol⁻¹). At 5-min intervals for 30 min, 0.5 ml of culture was transferred to 1 ml 10% trichloroacetic acid (TCA), boiled briefly and the acid insoluble radioactivity determined. The rate of incorporation was determined from the slope of the resultant curves compared with the rate of incorporation of the culture before the medium change, and plotted at the midpoint of the 30-min incorporation. Ribosomal RNA transcription (▲): cells were deprived of tyrosine as described above. At intervals, a 3-ml aliquot was mixed with 0.5 mCi of ³H (methyl) methionine. After 60 s the culture was chilled and the RNA extracted from broken cells and analysed on 2.75% polyacrylamide gels as described⁷. The radioactivity in RNA species was normalised to the absorbance of 25S RNA and compared with the value for a culture labelled before the medium change. Template activity (○): cells were deprived of tyrosine as described above. At intervals, 25-ml aliquots were chilled and total RNA prepared. The template activity of each preparation was determined at limiting RNA concentration¹², and compared with the activity for the RNA from the culture before the medium change.

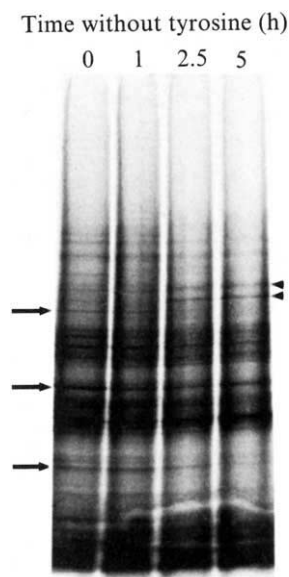
to the *rc* gene product and/or its synthesis of ppGpp, little is known about the detailed mechanisms involved in controlling the two types of transcription. To what degree does the stringent response exist in eukaryotic cells, which use separate polymerases for the transcription of ribosomal RNA and messenger RNA⁶? We⁷ and others⁸ have shown that in *Saccharomyces cerevisiae* deprived of an amino acid, the transcription of ribosomal RNA is severely inhibited. Ribosomal RNA transcription is also inhibited in cells treated with cycloheximide^{7,9}, but it continues almost normally in cells in which synthesis of ribosomal proteins has specifically been inhibited through a temperature-sensitive mutation¹⁰. We demonstrate here that in cells deprived of an amino acid there is a specific depletion of messenger RNA coding for ribosomal proteins.

The experiments are based on our ability to translate yeast mRNA quantitatively in a wheatgerm extract and to isolate the yeast ribosomal proteins produced¹¹. Total protein synthesis and the synthesis of yeast ribosomal proteins are linearly dependent on added yeast RNA, reaching a maximum at the same RNA concentration¹². Thus, unless a saturating concentration of yeast RNA is added to the wheatgerm extract, the radioactivity in any individual ribosomal protein spot is a measure of the relative amount of mRNA specific for that protein.

Cells were deprived of tyrosine as described in Fig. 1 and at intervals the rates of protein synthesis and ribosomal RNA transcription were determined. The effects of tyrosine deprivation on protein synthesis are complete only after an hour, presumably due to a substantial pool of tyrosine within the cells. The transcription of ribosomal RNA decreases slightly later than protein synthesis and levels off at a rate 15–20% of normal. On the other hand, the template activity of RNA prepared from cells deprived of tyrosine remains almost unchanged for 5 h (Fig. 1). As the half life of mRNA in yeast is between 6 and 30 min at 23 °C (ref. 13), substantial synthesis of new mRNA must continue, as was demonstrated directly elsewhere⁷.

Figure 2 is a one-dimensional polyacrylamide gel analysis of proteins synthesised by a wheatgerm extract in response to RNA from cells deprived of tyrosine. Although most proteins show

Fig. 2 Template activity of RNA from cells deprived of tyrosine. Cell-free translation was carried out with wheatgerm extract using as template RNA from cells deprived of tyrosine for increasing time, as described in Fig. 1. An aliquot was treated with 5% TCA, the precipitate electrophoresed on a 7.5–25% acrylamide gel in the presence of SDS according to the method of Laemmli¹⁴. Protein synthesis inhibition is indicated by the arrows, enhancement by arrowheads.



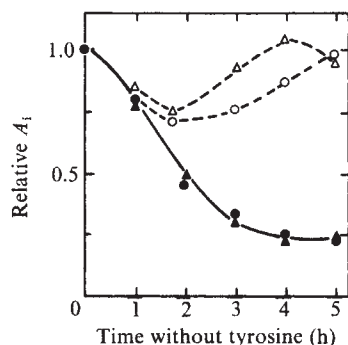


Fig. 3 Template activity for specific proteins. Aliquots of the cell-free translation described in Fig. 1 were subjected to two-dimensional polyacrylamide gel electrophoresis¹¹, and ribosomal and nonribosomal protein spots were cut out. The $A_i = (^{35}\text{S}/^3\text{H})_i / (^{35}\text{S}/^3\text{H})_{\text{total}}$ (ref. 11) was determined for each, and compared with the unstarved control. ●, Ribosomal protein no. 5; ▲, ribosomal protein no. 33; ○, nonribosomal protein C; △, nonribosomal protein D.

little change, the synthesis of some is inhibited (arrows) and that of others enhanced (arrowheads). Figure 3 shows a quantitative analysis of the template activity for individual ribosomal and nonribosomal proteins for the experiment described in Fig. 1. It is clear that as cells are deprived of tyrosine, they gradually lose mRNA for these two ribosomal proteins. That this is true of most, but not all, ribosomal proteins is shown in Table 1. Some of the exceptions, such as nos 12 and 14, are also exempt from the coordinate regulation in the *ts* mutants described above¹¹. The values for individual proteins in Table 1 and in other experiments are remarkably uniform between experiments, suggesting that the variation between proteins is due to slight differences in the stability of mRNA and/or regulation of its transcription. Nevertheless, the ribosomal proteins seem to be regulated as a class.

Table 1 Template activity for ribosomal and nonribosomal proteins

Protein no.	Subunit	Relative template activity at 5 h	
Ribosomal proteins		Expt 1	Expt 2
1	L	0.26	0.36
2	L	0.35	0.42
5	S	0.34	0.25
6	L	0.30	0.30
8	L	0.41	0.40
11	L	0.65	0.42
12	S	1.30	0.71
13	S	0.52	0.42
14	S	0.89	0.97
24	L	0.47	0.39
28	L	0.30	0.33
29	L	0.30	0.34
30	S	0.90	0.81
33	L	0.22	0.26
38	L	0.43	0.31
39	L	0.47	0.41
40	S	0.96	0.75
41	S	0.34	0.36
45	L	0.33	0.30
46	L	0.35	0.32
Nonribosomal proteins			
C		0.96	0.98
D		0.82	0.94
E		1.06	0.92
G		1.00	1.26

Template activity for ribosomal proteins and nonribosomal proteins was measured as described in Fig. 3. The values represent the ratio of template activity of RNA from cells deprived of tyrosine for 5 h to the template activity of RNA from unstarved cells, for two independent experiments. Experiment 2 is that of Fig. 3. L is the large (60S) subunit, S is the small (40S) subunit.

These results show that in yeast cells deprived of an amino acid there is a parallel inhibition in the synthesis of both ribosomal RNA and mRNA for most ribosomal proteins. The fact that the decline in mRNA activity occurs somewhat later than that of rRNA transcription may be due to the finite half life of mRNA for ribosomal proteins, which at 23 °C is probably 20–30 min^{13,15}. The kinetics in Figs 1 and 3 are consistent with the transcription of rRNA and mRNA for ribosomal proteins being inhibited simultaneously. It seems, therefore, that yeast has a fully coordinated stringent response to the deprivation of an amino acid, which involves ribosomal RNA as well as mRNA for ribosomal proteins, but excludes both tRNA and mRNA for the bulk of the cell's proteins⁷. Although ppGpp has not been detected in such conditions (J. Gallant, personal communication), a search for 'relaxed' mutants and for a regulatory molecule may be useful.

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***In vivo* aminoacylation and 'processing' of turnip yellow mosaic virus RNA in *Xenopus laevis* oocytes**

It is now well established that several plant and animal virus RNAs possess a 'tRNA-like' structure, as they are recognised *in vitro* by many tRNA-specific enzymes. To approach the problem of the possible physiological significance of these structures for the development of the virus, it seemed important to determine whether they are also recognised *in vivo*. The most logical approach would have been to use plant protoplast systems to study the behaviour of viral genomes in the infected cells. However, because such systems are still difficult to control, we decided to use the oocyte system. We describe here how turnip yellow mosaic virus (TYMV) RNA, which can be aminoacylated *in vitro* by valine¹, was microinjected into *Xenopus laevis* oocytes, incubated in the presence of ³H-valine and the RNA charged *in vivo* analysed. We conclude that TYMV RNA, the 3' terminal sequence of which is –CC (refs 2, 3), is aminoacylated *in vivo*. This indicates that the viral RNA is recognised by the oocyte tRNA nucleotidyltransferase and valyl-tRNA synthetase. Furthermore, the TYMV Val-RNA recovered after the *in vivo* reaction is fragmented and migrates between 4S and 5S RNA markers. This implies that (an) RNase(s) split(s) the viral genome, liberating this tRNA-like structure.

Table 1 Aminoacylation of total TYMV RNA, of TYMV coat protein mRNA or of *E. coli* tRNA^{Val} microinjected into *X. laevis* oocytes

Expt	(t)RNA injected	pmol* per oocyte	No. of oocytes injected	c.p.m. per oocyte
	Species			
1	None	—	40	1,546
	Total TYMV RNA	0.37	40	1,743
	<i>E. coli</i> tRNA ^{Val}	22	7	30,920
2	None	—	51	1,396
	Total TYMV RNA	0.37	53	1,509
	TYMV coat protein mRNA	0.17	56	1,130

TYMV was extracted from infected Chinese cabbage leaves according to Leberman¹². Total TYMV RNA was recovered using the phenol-chloroform-sodium laurylsulphate method^{13,14}. Partially purified viral RNA coding *in vitro* for coat protein (coat protein mRNA) was separated from genomic RNA by chromatography on a Sepharose 6B column. Fully grown oocytes from *X. laevis* females were preincubated for 3 h at 20°C in modified Barth's medium¹⁵ (100 µl per 10 oocytes) containing ³H-valine (31 Ci mmol⁻¹; Amersham) at a final concentration of 1 mCi per ml. They were microinjected¹⁶ with the following substrates in 50 nl of sterile water: 11.2 mg ml⁻¹ of *E. coli* tRNA^{Val} (Sigma), 15.4 mg ml⁻¹ of total TYMV RNA, or 870 µg ml⁻¹ of TYMV coat protein mRNA, and incubated at 20°C in fresh medium containing ³H-valine as above. After 2 and 24 h of incubation for experiments 1 and 2, respectively, the oocytes were washed with modified Barth's medium and stored at -70°C. They were homogenised and the RNA extracted as reported by Gatica *et al.*¹⁷. After ethanol precipitation, the RNA pellets were dissolved in sterile water and stored at -20°C. Aliquots were precipitated by 10% cold TCA, filtered through nitrocellulose membranes and the radioactivity retained measured in the presence of Bray's solution. Further analyses were carried out using the samples from experiment 2, except for the *E. coli* ³H-Val-tRNA^{Val}, which was from experiment 1.

* The pmol of (t)RNA injected are based on molecular weights of 2×10⁶ for total TYMV RNA, 0.25×10⁶ for TYMV coat protein mRNA and 25,000 for tRNA^{Val}.

To see if TYMV RNA could be charged *in vivo*, *X. laevis* oocytes were microinjected with total TYMV RNA (containing genomic RNA and coat protein mRNA)⁴, partially purified TYMV coat protein mRNA or *Escherichia coli* tRNA^{Val}. After 2 or 24 h of incubation in the presence of ³H-valine, the RNA was extracted from the oocytes and an aliquot used to determine the cold trichloroacetic acid (TCA)-precipitable counts; these are given in Table 1. No significant variation in counts is observed with oocytes injected with total TYMV RNA as compared to control, uninjected oocytes, even after 24 h of incubation. The same is true when TYMV coat protein mRNA is injected. Conversely, incubation with *E. coli* tRNA^{Val} leads to a 20-fold stimulation after only 2 h of incubation.

The fact that no increase in cold TCA-precipitable counts could be detected with the viral RNA preparations can certainly be explained by the low amount of TYMV RNA injected as compared to tRNA^{Val}. Indeed, the charging capacity of total TYMV RNA on a weight basis is about 80 times lower than that of a tRNA (see legend to Table 1).

Because it had not been possible to detect TYMV RNA charging by TCA precipitation, it was necessary to analyse the size of the ³H-Val-oligonucleotides recovered after T₁ RNase digestion of the RNA extracted from the oocytes. This was carried out by paper electrophoresis in 0.5 M formic acid (results not shown) or by thin-layer chromatography (TLC) on polyethyleneimine-cellulose (PEI-cellulose) plates (Fig. 1).

The T₁ digests of TYMV Val-RNA charged *in vitro* reveal the presence of a Val-pentanucleotide (Fig. 1, sample 4) and those of *E. coli* Val-tRNA, also charged *in vitro*, of a Val-pentanucleotide and a Val-tredecannucleotide (sample 6). This agrees with the known sequence of the 3' region of TYMV RNA^{2,5,6} and of the valine-specific *E. coli* tRNAs^{7,8}, respectively.

The endogenous oocyte tRNAs charged *in vivo* (sample 1, see also samples 2 and 3) produce two Val-oligonucleotides of about eight and nine residues, plus a third spot which migrates faster than a pentanucleotide.

The analyses of the Val-oligonucleotides obtained after T₁ digestion of the RNAs extracted from oocytes injected either by total TYMV RNA (sample 2) or TYMV coat protein mRNA (sample 3) showed in each case the presence of a Val-pentanucleotide, demonstrating the *in vivo* aminoacylation of TYMV RNA. This ³H-Val-pentanucleotide is much less intense when the oocytes are injected with TYMV coat protein mRNA than with total TYMV RNA, and this is probably due to a partial degradation of the RNA molecules during the purification procedure.

In sample 2, the Val-oligonucleotides of about eight and nine residues migrate somewhat faster than those in samples 1 and 3, and this is probably due to the presence of salts in sample 2. In samples 1, 2 and 3 (*in vivo* charging experiments) a spot appears that migrates faster than the Val-pentanucleotide. Although this spot is not produced when total oocyte tRNAs are charged *in vitro* (results not shown) by an *E. coli* extract or by a rat liver extract, one cannot exclude the possibility that it corresponds to a tRNA isoacceptor for valine only charged by the homologous enzyme. This supplementary spot was shown not to be due to free valine, Val-AMP or Val-adenosine (data not shown).

After injection of *E. coli* tRNA^{Val} into the oocytes (sample 5), an ³H-Val-tredecannucleotide appears which corresponds to the T₁ digest of ³H-Val-tRNA^{Val}. No spot corresponding to the digests of endogenous oocyte ³H-Val-tRNA is observed. This is due to the fact that the same number of counts were deposited in all cases shown in Fig. 1, and that after injection of *E. coli* tRNA^{Val}, a 20-fold stimulation in cold TCA-precipitable counts

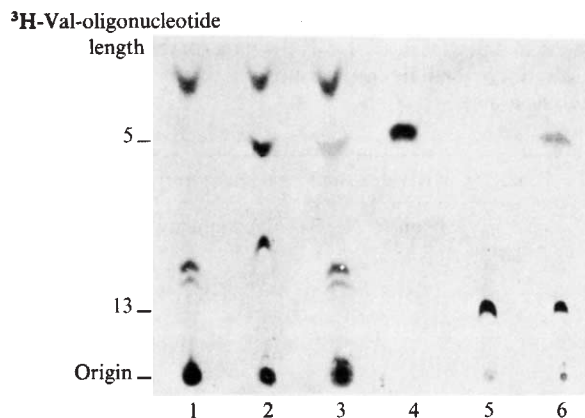


Fig. 1 Separation by TLC on PEI-cellulose plates of the ³H-Val-oligonucleotides obtained after T₁ RNase digestion of *in vivo* or *in vitro* charged (t)RNAs. The *in vitro* aminoacylation with ³H-valine (31 Ci mmol⁻¹; CEA) of unfractionated *E. coli* tRNAs or of total TYMV RNA was carried out as described by Yot *et al.*² using an *E. coli* extract devoid of tRNAs. ³H-Val-(t)RNAs (20,000 c.p.m.) recovered after *in vivo* (see Table 1) or *in vitro* aminoacylation were incubated for 45 min at 37°C in a mixture (15 µl) containing 20 mM sodium citrate, pH 5, 1 mM EDTA, and 0.25 units of T₁ RNase (Sankyo) per µg of Val-(t)RNA. The PEI-cellulose W.R. plates (20×20 cm, plastic-backed, with luminescer, Schleicher and Schüll) were soaked in methanol, dried and the digests applied. The plates were developed at room temperature in a stepwise fashion without intermediate drying, using a slight modification of the method of Gupta and Randerath¹⁸: with water to 1.4 cm above the origin; 0.05 M Tris-HCl, pH 6.8, to 4.2 cm; 0.15 M Tris-HCl-8.5 M urea to 7.7 cm; 0.3 M Tris-HCl-8.5 M urea to 10.5 cm; 0.5 M Tris-HCl-8.5 M urea to 14 cm, and finally, with 0.75 M Tris-HCl-8.5 M urea to 16.8 cm. The sheet was dried, fluorographed^{19,20}, and exposed at -70°C using Kodak X-Omat R films. The T₁ digests correspond to the ³H-Val-oligonucleotides obtained from: 1, uninjected oocytes; 2, oocytes injected with total TYMV RNA; 3, oocytes injected with TYMV coat protein mRNA; 4, *in vitro* charged TYMV RNA; 5, oocytes injected with *E. coli* tRNA^{Val}; 6, *in vitro* charged *E. coli* tRNAs.

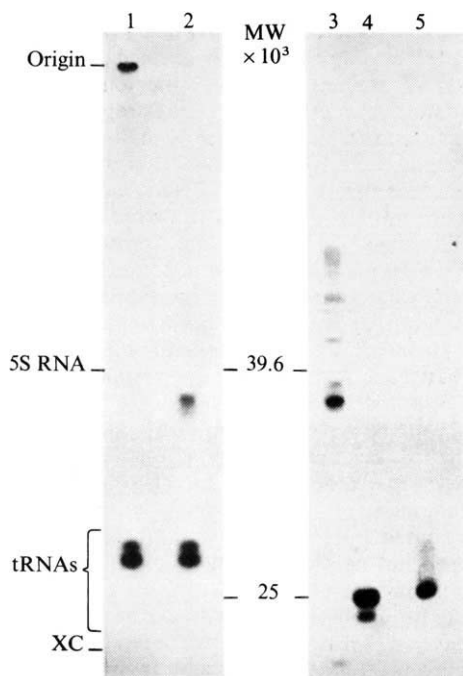


Fig. 2 Analysis by 10% polyacrylamide-7 M urea gel electrophoresis of the TYMV ^3H -Val-RNA charged *in vivo*. Polyacrylamide gel electrophoresis was a modification of the method of Donis-Keller *et al.*²¹; the sample buffer, polyacrylamide gel and electrophoresis buffer were the same but at pH 7, and the Val-(t)RNA samples (10,000 c.p.m.) were not heated before the run. Electrophoresis was carried out at 4°C and 200 V, and the electrophoresis buffer was constantly recycled. The gel was soaked in 10% cold TCA for 1 h, fluorographed^{20,22} and exposed at -70°C. Marker RNAs were run in parallel and stained with ethidium bromide. 1, Uninjected oocytes; 2, oocytes injected with total TYMV RNA; 3, *in vitro* charged TYMV RNA; 4, oocytes injected with *E. coli* tRNA $^{\text{Val}}$; 5, *in vitro* charged *E. coli* tRNAs. XC, xylene cyanol FF.

was observed (Table 1) as compared to uninjected oocytes. Consequently, the corresponding products from endogenous oocyte tRNAs are not visible.

The T_1 digests of the samples presented in Fig. 1 were also separated by paper electrophoresis in 0.5 M formic acid; the conclusions reached agree with those of Fig. 1, namely, that TYMV RNA is aminoacylated when injected into the oocytes.

TYMV RNA as extracted from the virions terminates at the 3' end by -CC. Thus, a positive response to valine aminoacylation indicates that the RNA is recognised *in vivo* by the oocyte tRNA nucleotidyltransferase as well as by the valyl-tRNA synthetase. Solari *et al.*⁹ reached similar conclusions when testing the aminoacylation of injected tRNA $^{\text{Phe}}$ devoid of its 3'-terminal adenosine.

It was important to determine the size of the TYMV Val-RNA charged in the oocytes. To this end, ^3H -Val-(t)RNAs charged *in vivo* (oocytes) or *in vitro* (*E. coli* extract) were analysed by electrophoresis on a 10% polyacrylamide, 7 M urea gel (Fig. 2). Total TYMV RNA charged *in vivo* (slot 2) shows in addition to endogenous Val-tRNAs (slot 1) a Val-RNA band migrating between 4S and 5S markers and corresponding to the tRNA-like fragment of TYMV. In the case of TYMV RNA charged *in vitro* (slot 3), an intense band migrating in the same position appears as well as a series of fainter bands of higher molecular weight. Experiments of *in vitro* aminoacylation of TYMV RNA indicate that, with prolonged incubation, these bands of higher molecular weight give rise to the tRNA-like fragment (S. J. and A.L.H., unpublished results).

The *E. coli* system is known to contain RNase P, a maturation enzyme of tRNAs (ref. 10). Extracts containing this enzyme

have been shown^{6,11} to split TYMV (Val)-RNA, releasing a 4-5S fragment from the 3' end of the genome (see also Fig. 2, slot 3). The experiments presented here strongly suggest that (a) similar enzyme(s) exist(s) in the oocyte.

As a further control, oocytes were injected with tRNA $^{\text{Val}}$ (slot 4); a main band corresponding to Val-tRNA was obtained, and similarly by *in vitro* charging of total *E. coli* tRNAs (slot 5).

The results presented here demonstrate that the tRNA-like structure of a viral RNA is recognised *in vivo* by enzymes specific for tRNAs. Furthermore, they show that TYMV RNA is 'processed' *in vivo*, liberating its tRNA-like structure.

Even though these results were obtained using an animal cell, it is likely that similar conclusions would be reached with a plant system. As plant cells are not yet accessible for such studies, the oocyte might constitute a useful material for the study of the physiological role of tRNA-like structures in viral genomes. In the first place, it is tempting to speculate that the 4-5S RNA fragment formed *in vivo* plays a part in the infected cell by inhibiting, for example, a factor involved in cellular protein synthesis. It is not unlikely that in the infected plant cell a part of the viral genome could be sacrificed for the formation of such an inhibitor. Indeed, preliminary results (S.J. and A.L.H. unpublished observations) with plant extracts indicate that they also contain (an) enzyme system(s) capable of liberating the tRNA-like structure from TYMV RNA.

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Relationship of protein thermostability to accessible surface area

THE pH of an unbuffered protein solution subjected to a continuous temperature increase usually exhibits a single inflection point associated with the cooperative thermal denaturation of the native protein structure¹. Bull and Breese² have used this procedure to demonstrate that the midpoints (T_m) for the thermal denaturation of 14 proteins correlate with the average residue volumes of their constituent amino acid residues and have proposed that this parameter is the principal determinant of protein thermostability. We report here our study of their proposal by extension of these measurements to include 20 additional well characterised proteins, selected because of their availability in homogeneous form. With this expanded data base, solvent-accessible surface area was found to result in a better correlation with protein thermostability.

The apparatus used is similar to that described by Bull and Breese¹, a Radiometer model 25 pH meter, a Radiometer GK 2401C combination electrode, a model 425C (Yellow Springs) bridge and thermistor probe, and an Omniscribe dual pen recorder (Houston). Individual protein solutions of about 2 mg ml^{-1} concentration were dialysed against 0.2 M KCl before use. The protein solution, pH electrode, thermistor probe and a small magnetic stirring bar were inserted into a jacketed vessel surrounded by a grounded wire screen. The temperature of the protein solution was continuously varied by a circulating water bath programmed to increase by 0.83°C per min. Observed pH values were corrected for temperature as described by Goodno *et al.*³.

The T_m values for 30 proteins are listed in Table 1 in increasing value. Each protein preparation used exhibited a single

Coomassie blue staining component when examined by polyacrylamide gel electrophoresis in the presence of detergent, and a single $\Delta pH/\Delta T$ discontinuity over the temperature range $30\text{--}90^\circ\text{C}$. The single exception to this is the preparation of the S1 fragment of myosin, which contains a major component of molecular weight 9.3×10^5 . Scanning calorimetric measurements indicate that this component has a T_m of 43°C . The T_m values listed are the highest values measured in the pH range $4\text{--}9$. Those listed in Table 1 for nine proteins (6,7,21,22–25,27,30) of the 10 proteins studied in common with Bull and Breese have T_m values agreeing with those previously reported indicating that our execution of the $\Delta pH/\Delta T$ procedure is appropriate. However, our measurements do not support the correlation between T_m and average residue volume (V_R) reported by Bull and Breese². In contrast to their correlation coefficient of $+0.96$, we find a value of -0.02 . Similarly, in contrast to their correlation coefficient between T_m and hydrophobic index of $+0.62$, we find a value of -0.01 . We conclude that the previously reported strongly positive correlation coefficients resulted from examination of a limited number of proteins and do not necessarily reflect any fundamental principle of protein stability.

In searching for an alternative explanation for the observed variation in protein thermostability, we noted that T_m exhibited an imperfect inverse trend with polypeptide chain length. As the ratio of surface to volume for globular proteins decreases with chain length, we have related the observed T_m values to the fractional area of the polypeptide accessible to the solvent. The total surface area of a denatured polypeptide chain, A_t , was calculated from the relationship⁴ $A_t = 1.44M$, where M is the molecular weight of the protein; the solvent-accessible surface area of an individual protein globular domain was calculated using the relationship⁵ $A_s = 11.116M^{2/3}$, and the accessible surface area of a globular domain in a dimeric or square planar tetrameric polymer of identical globular domains was deter-

Table 1 Protein parameters

Code	Protein	Source	pH	T_m	V_r	Φ	$\log M_s$	n
1	Myosin S1 fragment	Rabbit muscle	6.83	43 ± 1	87.6	788	4.97	2
2	Phosphorylase	Rabbit muscle	8.06	48 ± 2	88.9	886	4.97	4
3	Cytochrome <i>c</i> peroxidase	Yeast	6.49	50 ± 1	87.1	831	4.53	1
4	Fumarase	Porcine heart	6.91	53 ± 1	87.1	839	4.68	4
5	β -galactosidase	<i>Escherichia coli</i>	7.48	54 ± 1	86.1	826	5.13	4
6	Trypsinogen	Bovine pancreas	5.80	55 ± 3	79.7	770	4.39	1
7	Chymotrypsinogen	Bovine pancreas	4.90	56 ± 1	80.2	792	4.41	1
8	Lactate dehydrogenase	Rabbit muscle	6.86	56 ± 1	85.7	908	4.56	4
9	Phosphoglycerate kinase	Yeast	7.01	56 ± 3	84.4	832	4.69	1
10	Elastase	Porcine pancreas	6.70	57 ± 1	82.2	799	4.41	1
11	Carbonic anhydrase	Bovine erythrocyte	6.59	57 ± 2	86.2	829	4.47	1
12	Aldolase	Rabbit muscle	6.51	58 ± 1	84.3	792	4.60	4
13	Triosephosphate isomerase	Rabbit muscle	7.39	61 ± 1	82.3	737	4.43	2
14	Catalase	Bovine liver	6.92	61 ± 2	86.3	805	4.73	4
15	Pyruvate kinase	Rabbit muscle	6.97	62 ± 1	84.5	793	4.76	4
16	Adenylate kinase	Rabbit muscle	6.69	62 ± 2	86.0	793	4.33	1
17	Carboxypeptidase	Bovine pancreas	6.69	63 ± 1	86.1	657	4.53	1
18	Alcohol dehydrogenase	Horse liver	7.12	64 ± 2	82.7	830	4.61	4
19	Subtilisin	<i>Bacillus subtilis</i>	6.22	65 ± 2	76.9	739	4.44	1
20	Serum albumin	Bovine plasma	6.40	65 ± 3	86.9	838	4.82	1
21	Haemoglobin	Bovine erythrocyte	6.10	67 ± 1	84.3	873	4.20	4
22	Concanavalin A	Jack bean	6.56	71 ± 2	82.8	846	4.41	4
23	Lysozyme	Hens egg	6.50	72 ± 1	83.0	706	4.16	1
24	Myoglobin	Whale muscle	6.40	76 ± 2	87.8	850	4.25	1
25	Ovalbumin	Hen's egg	6.47	76 ± 2	86.1	866	4.64	1
26	Trypsin inhibitor	Soy bean	7.61	77 ± 1	85.2	878	4.05	1
27	Lactalbumin	Bovine milk	4.50	82 ± 3	88.1	939	4.15	1
28	β -lactoglobulin	Bovine milk	6.10	83 ± 1	88.2	869	4.27	2
29	Superoxide dismutase	Bovine erythrocyte	7.15	83 ± 1	79.0	675	4.20	2
30	Cytochrome <i>c</i>	Horse heart	7.80	83 ± 2	87.0	742	4.10	1

Columns headed by the terms or symbols, pH , T_m , V_r , Φ , $\log M_s$ and n refer to the pH at which the T_m was observed, the T_m measured by the $\Delta pH/\Delta T$ procedure, the average residue volume, the hydrophobic index, the log of the polypeptide molecular weight and the number of polypeptide chains present in the native protein, respectively. The hydrophobic index was calculated as described by Bigelow¹¹.

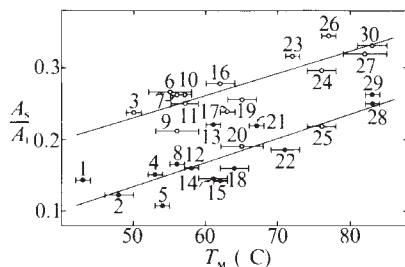


Fig. 1 Relationship of fractional polypeptide area accessible to solvent, A_s/A_t , and protein thermostability, T_m . A_s is the polypeptide surface area in $\text{\AA}^2$ accessible to the solvent in a native globular protein, A_t the total accessible surface area of a denatured protein, and T_m the midpoint of the thermal denaturation detected by the $\Delta pH/\Delta T$ procedure. The open circles indicate monomeric proteins and the filled circles polymeric proteins. The number associated with each experimental value indicates the identity of the protein as encoded in Table 1. The lines represent least-squares analyses of the values for the monomeric (except for proteins 20 and 25) and the polymeric proteins.

mined using the factors⁵ 0.857 and 0.714, respectively, to account for the surface lost in the monomeric unit by polymerisation. As seen in Fig. 1, the values for the 30 proteins seem to cluster about two linear relationships with a common slope, one relationship being populated exclusively by monomeric proteins and the second principally by polymeric proteins.

The slopes of these lines suggest that increasing the internal area of these proteins at the expense of surface area decreases the thermostability in the temperature range considered. As hydrophobic index and percentage apolar atoms are independent of polypeptide chain length, and as polar atoms are preferentially located on protein surfaces, it follows that more polar atoms, particularly those of the peptide backbone, must be internalised as the fractional surface area is diminished. Such internalisation of polar atoms in contrast to apolar atoms presents a problem, for burial of a polar atom not complementarily hydrogen bonded in the internal apolar environment is energetically unfavourable. We propose that as more polar atoms need to be internalised to fold longer polypeptide chains into globular units, the probability of noncomplementary burial increases, leading to the decreased thermostability indicated in Fig. 1. In the case of burial of peptide backbone polar atoms, it should be noted that the amount of secondary structure, a common method to pair these atoms complementarily, is independent of chain size. The relationship exhibited by the monomeric proteins predicts that a polypeptide with a molecular weight $>60,000$ folded into a single globular domain would be unstable when present in an organism maintained at 37°C . Fisher⁶ has suggested that large polypeptide chains would be stabilised by forming asymmetrical structures, thus increasing their surface to volume ratios.

Alternatively, the results shown in Fig. 1 suggest two alternative ways in which the thermostability of larger polypeptide chains can be enhanced. One involves using globular surfaces to contribute to conformational stability through association of globular subunits to form polymers. Analysis⁷ of subunit interfaces suggests that polar complementarity provides the selectivity while apolar interactions provide the added stability for these inter-subunit interactions. The relationship exhibited by the polymeric proteins in Fig. 1 suggests that polypeptide chains with molecular weights up to about 160,000 would be stable at 37°C provided that such chains fold into globular units and that the globular units polymerise. This limitation would encompass the polypeptide chain lengths of all globular polymeric proteins⁸. The results shown in Fig. 1 also suggest a second way to increase the thermostability of larger polypeptide chains, namely, folding a single chain into multiple globular domains. A case in point is the apparent enhanced thermostability of phosphoglycerate kinase, protein 9. The crystallographic structure⁹

of this protein demonstrates that its single polypeptide chain folds into two globular domains of nearly equal size, with minimal interdomain contact; this conformation would increase the fractional surface area relative to folding into a single globular domain and accordingly increase thermostability. Analysis¹⁰ of the sequence of serum albumin suggests that this protein folds into three globular domains of closely related sequence, probably the result of fusion of duplicated structural genes. The position of the T_m for serum albumin, protein 20 in Fig. 1, suggests that its structure resembles that of a covalently linked polymeric protein. The position of the T_m for ovalbumin, protein 25, in Fig. 1, suggests that its relatively large polypeptide chain is also folded into more than one globular domain. Unfortunately, no sequence or crystallographic information is yet available for ovalbumin to validate this suggestion.

In presenting our analysis of thermostability in terms of the size of globular domains and polymerisation of domains, we recognise that other considerations such as the presence of disulphide bonds, ligation of strongly bound metal ions, haem moieties and other cofactors, packing densities, and intra-surface interactions will also influence thermostability. Indeed, such considerations may be partly responsible for the scatter of the values about the linear relationships considered in Fig. 1. We also recognise that the inverse A_s/A_t dependence on T_m for globular proteins produces an absurd extrapolation, namely, that the most thermostable protein would have no buried area. Clearly, other considerations must limit this relationship. However, it seems to extend to include pancreatic trypsin inhibitor, which has a A_s/A_t value corresponding to a T_m of 111°C , as no $\Delta pH/\Delta T$ discontinuity could be detected for this protein at temperatures up to 98°C . Although, as more information becomes available, our analysis of thermostability may suffer the same fate as the average residue volume correlation, a cursory review of thermal inactivation measurements for a variety of enzymes suggests that size of globular domains and polymerisation are critical to protein thermostability.

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Errata

In the letter 'The units of calcium conduction in *Helix* neurones' by N. Akaike *et al.*, *Nature* **274**, 379–382, ref. 6 should read: Kostyuk, P. G., Krishtal, O. A. & Pidoplichko, V. I. *Nature* **257**, 691–693 (1975). (This replaces the reference to a 1977 paper by the same authors.)

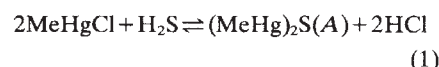
In the letter 'Single crystal analysis of the structure of stishovite' by W. Sinclair and A. E. Ringwood, *Nature* **272**, 714, in paragraph 4 line 2 the space group should read: $P4_2/m2_1/n2/m$.

In the letter 'Origin of pregalactic microwave background' by M. J. Rees, *Nature* **275**, 35–37, in paragraph 1 lines 5, 6 should read: $(\delta\rho/\rho) \propto M^{-\alpha}$ ($\frac{1}{3} \leq \alpha \leq \frac{2}{3}$) (refs 12–15). Also, in paragraph 6 lines 1, 2 should read: Nucleosynthesis could have supplied heavy elements by $t \approx 10^7$ yr.

matters arising

Volatilisation of methylmercuric chloride by hydrogen sulphide

TREATMENT of methylmercuric chloride with hydrogen sulphide in aqueous solution has been shown by Rowland *et al.* to result in volatilisation of mercury¹. The volatile product was not identified but was considered to be a 'volatile sulphur compound of methylmercury'. Volatilisation of mercury and concurrent lowering of the pH of the aqueous solution¹ can be explained by formation of bis(methylmercuric) sulphide (A).



This reaction has previously been carried out in ethanol^{2,3}. The foul odour of A (ref. 2) indicates that it is volatile, even though it is a solid at room temperature. Loss of mercury caused by ammonium sulphide¹ can also be attributed to formation of A.

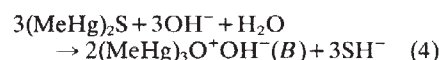


The reduced effectiveness of this reagent compared with hydrogen sulphide¹ can be explained by the simultaneous occurrence of reaction (3).

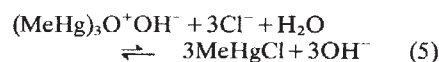


Reactions analogous to (2) and (3) have been observed between MeHgBr and Na₂S (refs 4, 5).

Absorption of the volatile mercurial by alkali¹ can be attributed to formation of the known⁶ tris(methylmercuri)oxonium hydroxide (B) (contrast with doubtful existence of MeHgOH⁶).



The ionic character of B would explain the failure of the base-soluble species to undergo chromatography¹. Although chloride ions, from absorption of volatilised HCl, would also be present, the equilibrium (5) would lie well to the left. However, extraction with toluene would remove the non-ionic methylmercuric chloride and drive the equilibrium to the right, explaining recovery¹ of MeHgCl by this method.



The volatilisation of methylmercury could also be explained by formation (6)



of MeHgSH (reported preparations^{7,8}), and reactions (2) and (4) could be modified to accommodate this. However, reaction (6) has not previously been demonstrated, whereas there is precedent for (1)–(3)^{2–5}, hence A is preferred as the source of volatility. Since A is unstable, decomposing by (7)², dimethylmercury and mercuric sulphide would probably be the ultimate products from MeHgCl and H₂S in an environmental situation.



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World's oldest animal traces

BIOLOGISTS and biogeologists would like to know when multicellular animal life began. Well authenticated records show that it was present by ~680 Myr ago, but older records have not withstood scrutiny. Clemmey's report¹ of metazoan burrows from 1,000 Myr old rocks in Zambia therefore excited great interest. I have examined the specimens and seen Clemmey's coloured slides of the outcrops, and this examination and subsequent research led to a different conclusion from his. The Zambian burrows are undoubtedly metazoan and indigenous to the sedimentary deposits in which found, but they are of post-depositional origin and related to a geologically recent surface. Evidence for this conclusion rests in the bleached, saprolitic nature of the host rock and the contrasting red and pink banded burrow-fillings, containing rounded, ferromanganese-coated quartz grains that are much larger than particles of the adjacent matrix. Such characteristics suggest introduction of burrow-filling material from a relatively recent lateritic weathering surface.

Further study and inquiry among students of the social insects led to the additional conclusion that Clemmey's burrows are the work of termites, digging through and parallel to the softer layers of the steeply dipping saprolitic rock to obtain water for humidification of their nests. From publications and correspondence with termite specialists M. Lüscher, Ch. Noirot, M. L. Roonwal and W. A. Sands, I found that termites do burrow to depths of 70 or more metres to reach the water table, that they burrow preferentially in the least resistant parts of the rock and that they backfill unused burrows with surface matter producing laminated burrow-walls and a banded 'spreite' pattern like that illustrated by Clemmey. His observation that the burrows are parallel or nearly so to the bedding, and that they favour particular stratigraphic levels, would be consistent with descent along the softer of the steeply dipping strata of the Zambian Copperbelt.

The above comments apply mainly to burrows of the type of Clemmey's Fig. 1. The oval profile, pinching and swelling along their lengths, and terminal branching of such burrows is also termite-like. The bioturbation of his Fig. 2 may represent a zone of termite foraging or collapse. Specimens like his Fig. 4 (with a hollow interior) resemble the covered passages through which termites cross lighted areas.

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CLEMMEY REPLIES—I agree that the burrows I described are only obvious after weathering, but this is a feature shared by much of the internal fabric of rocks and in this case has an explanation. The red and pink banding results from weathering of the microchemical/mineralogical differences between bioturbated and non-bioturbated sediment. Within the burrows it reflects the differences between sediment passed around the body or through the gut of the animal and such differences are to be expected in bioturbated sediment¹.

The burrow, as a microreducing environment, attracted the precipitation of iron sulphides, which ideally would be more concentrated in the zones of the bioturbated sediment richest in organic matter. On weathering the ferrous iron is oxidised to the ferric state and hence the

red and pink shades. The country rock depending on its iron content weathers cream, brown, or pink. Within the bioturbated sediment and sometimes in the country rock are pools of authigenic quartz such as may be formed when silicates are breaking down under biogenic attack or during weathering and are releasing large amounts of silicic acid. These are the 'quartz grains' referred to by Cloud.

If the burrows were made by termites, why did those making type A (ref. 2) burrows live only above the 2 m of strata at the base of the Orebody Member whilst the succeeding 2 m are characterised only by those making type B burrows? And why is the unit containing the burrows overlain by hundreds of metres of identical lithologies which show no signs of bioturbation. I believe that the burrows are not those of termites nor are they lithologically controlled, but they are paleofacies-controlled. The transition type A to type B burrows reflects the transition from intertidal to lagoonal/lacustrine sedimentation, as indicated by facies studies³.

Total destratification, such as indicated by my Fig. 2 (ref. 2), produces a swirled fabric in the rock; this is explained only by invoking contemporaneous sedimentation and bioturbation. For while small tunnels of termite origin are at least conceivable, complete fluid mixing over a thickness of several centimetres is not. This fabric implies that the medium undergoing bioturbation was granular and wet.

The idea that type B burrows represent covered passageways is not feasible. The burrows are an integral part of the rock, are not confined to exposed bedding surfaces and can be revealed by excavating the strata. They have pellet-formed walls which may show disintegration at the burrow termination into equidimensional pellets. Again these are part of the rock and not surficial features.

As far as it is possible to tell, the burrows (unequivocally metazoan according to Cloud) were formed contemporaneously with the sedimentation of the containing units about 1,000 Myr ago.

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No paradox in the control of energy intake

As Rothwell and Stock¹ note their results refute the notion that a particular size of energy store precisely controls energy

intake in rats. Other reports²⁻⁴ agree with this. However, contrary to what is claimed, their results do not necessarily reveal a paradox or physiological enigma in the control of energy intake in the rat. They are clearly compatible with the theories of Kleiber⁵, Adolph⁶, Ugolev⁷ and Booth⁸, in which energy metabolism controls feeding and the energy store is only one component in the feedback system. Rothwell and Stock state that our model⁹ has yet to include effects of body energy increments. On the contrary, in a publication which they cite¹⁰, and elsewhere¹¹, we have included a representation of effects of energy store size on intake through energy metabolism. We also emphasised that although the energy store may settle at a particular size¹¹⁻¹³, there is no privileged size either achieved or used as a homeostatic set-point. In these simulations the precision of defence and (if a specific size is maintained) the value defended depends on a compromise between the small but persistent graded effect of store size on metabolism and all the other dietary and endocrine conditions affecting metabolism.

Rothwell and Stock also state that it is difficult to conceive how such a model could explain an increase in fat deposition rather than a decrease in intake when energy supply is in excess of requirements. However, our model probably provides several ways of increasing efficiency without altering intake. For example, a change in regimen might divert a higher proportion of absorbed energy into fat, while also reducing the faecal excretion of energy or the thermic effect of feeding. All these effects would be liable to increase the size of the energy store in the model. The extra metabolism from the increased retention of energy intake would help reduce intake, and the extra diversion after absorption would help to increase it.

The importance of Rothwell and Stock's data lies in the questions raised as to the exact mechanism(s) by which dietary changes can alter the relations among the processes of utilisation, storage and intake. Quantitative simulation is of similar importance.

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ROTHWELL AND STOCK REPLY—We apologise to Booth and Toates if we have neglected those aspects of their feeding model that take into account energy storage. Nevertheless, we were well aware (and said so) that the increased fat deposition of our animals results from an increased metabolic efficiency (almost certainly due to a decreased thermic effect of feeding), thus providing a larger fraction of total ingested energy available to metabolism. According to Booth and Toates, this excess energy would either reduce total energy intake or, if diverted to fat storage, help to increase intake. In fact, we found that total energy intake remained at precisely the same level as that in the free-feeding control animals.

We would also suggest that an energostatic approach fails to account for the differences between the two types of energy load used in our experiments, that is, fat and Complan. If one accepts Booth's proposal¹ that it is the availability of energy that determines intake, it is logical to assume that isoenergetic stomach loads will have similar effects on voluntary energy intake. In our experiments, however, rats tube-fed 34% of normal intake precisely compensate for the load when it is a balanced nutrient mixture, but over-eat by 15-16% when the load is comprised of fat. This suggests that an energostatic approach may be too simplistic and, since our original letter was published, Savory has written to us supporting this view. He obtained very similar results to ours by feeding quail with diets of varying energy density², and one of his conclusions from this work was that "... differences in energy expenditure were not balanced by differences in nutrient intake, so perhaps the quail were regulating food intake to meet their requirements for some dietary component other than energy".

There are many attractive aspects of the energostatic model of Booth and Toates, and we regret that they did not take advantage of their computer simulation to substantiate the claim that our results are compatible with their feeding model. We hope that they will do so and if necessary, as we suspect it will be, make appropriate alterations.

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Induced suction and lithospheric plate pull

SHOEMAKER¹ proposes to explain the driving force of plate tectonics as 'induced suction' by the low density sediments in the trench. However, his calculation, which is based solely on fluid statics, does not tell us what the energy source for the motion is, neither does it tell us anything essentially new about subduction dynamics.

To illustrate this, consider an experiment in which a high viscosity fluid is flowing steadily over the edge of a horizontal table. The fluid which has just passed over the edge responds to the unbalanced vertical force of gravity, and this force is 'communicated' to the rest of the fluid by means of a horizontal pressure gradient (that is, the upper surface of the fluid is no longer horizontal). A statics calculation of the type made by Shoemaker would demonstrate that the table edge provides 'suction', but this obscures the fact that the cause of the motion is the vertical force of gravity. The essentials of the argument still apply if the rheology is more complicated.

In the plate tectonics context, the value Shoemaker obtains for the 'suction' is nothing more than the downward force on the subducting slab, presumably caused by gravity². Trench depression can be predicted by a dynamical calculation³, and the accumulation of sediments at the trench is an effect of plate motion, not a cause.

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1. Shoemaker, E. M. *Nature* **272**, 241 (1978).
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SHOEMAKER REPLIES—Induced suction¹ is a consequence of the combination of two conditions being satisfied by the subduction process: first, that the fluids or materials above and below the subducting plate have different densities and second, that the subducting plate bends beneath the overriding plate in contrast to the idealised situation where it passes from the horizontal to the downward dipping state through a sharp corner². The force of induced suction is not the downward force of the subducting slab nor is there any direct connection between these two forces. There is an obvious indirect connection in that the downward force of the subducting slab has a major influence in creating the downward bend which causes the induced suction.

The major energy source for plate motion may be downward pull of the subducting slab; it might be something else. However, any consideration of

energy is immaterial to the argument presented for 'deriving' the existence of induced suction. The argument was based on equilibrium which has equal validity with energy principles.

There is no connection between the example of a highly viscous fluid flowing over the edge of a table and induced suction. The 'communication' between the downward flowing fluid and the horizontal flowing fluid on the table is provided by the table edge; the horizontal component of the force exerted by the table edge upon the fluid pulls the fluid along the table. This is not induced suction. Moreover, the fluid will certainly flow if, for example, there is air above and below the fluid 'slab'.

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1. Shoemaker, E. M. *Nature* **272**, 241 (1978).
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Circadian variation in immune response to oxazolone

FACHET AND ANDO¹ suggest that cellular and humoral responses to oxazolone are under the control of *H-2* genes. Their evidence for the control of cellular immunity was based on the differences between inbred and *H-2* congenic strains of mice in the magnitude of the delayed hypersensitivity response to oxazolone. This was measured in the ears of sensitised mice as the difference in ear thickness measured immediately before and 24 h after challenge. Using this method, we have demonstrated in rats that the magnitude of the maximum immune response depends on the time of day at which oxazolone is applied to the ears². Subsequently, we have shown that there are circadian variations in the skin response to the injection of purified protein derivative in man³. It seems that the time of day at which the antigen is encountered influences the magnitude of the delayed hypersensitivity response.

Most investigators carry out experiments at the same time of the day. We would welcome an assurance from Fachet and Ando on this point, for if their experiments were not standardised to one time of challenge for all the mouse strains, this affects the interpretation of their findings. Additionally, if the oxazolone challenges were given over a long time span within a single day this might affect the results adversely, as the maximum observed response to challenge with oxazolone at 10.00 in the rat is over eight times the minimum response at 16.00.

Data on the time of challenge and the lighting regimen of the animals are not available in the paper from the Hungarian Unit which therefore does not contain sufficient details for the experiments to be repeated, surely a prerequisite of any scientific publication.

Biological rhythms influence many cell-mediated and humoral immune processes with effects which are often of a high order of magnitude. Our recent finding (unpublished) that the delayed hypersensitivity response to oxazolone can be manipulated by altering the lighting regimen of the animals is a further demonstration that the basic principles of chronobiological experimentation need to be introduced immediately into immunological studies.

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FACHET AND ANDO¹ REPLY—The points raised by Pownall *et al.* are of interest and we make the following comments. All experimental mice of the *H-2* congenic strains used were sensitised and their delayed-type hypersensitivity (DTH) to oxazolone was tested between 09.00 and 11.00. The same timing of experimental procedures was applied when the DTH to oxazolone was tested in a larger number of animals of the same strain and in mice of other inbred mouse strains. The same conclusion was reached concerning the effect of genes within and outside the *H-2* complex. A single strain has not been independently tested; a series of different strains only has been investigated and used for comparison¹.

Within the scope of our experiments we found no diurnal variation in responsiveness on testing *H-2* congenic mouse strains with oxazolone but it well might occur in different conditions.

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reviews

Rationality and actions

A Rational Animal, and Other Philosophical Essays on the Nature of Man. By Anthony Flew. Pp. 245. (Clarendon/Oxford University Press: Oxford, 1978.) £5.95.

THE attempt to demonstrate on *a priori* grounds that natural science can never fully explain human behaviour has been a fashionable parlour game amongst philosophers for some time. This question—and associated problems hinging on the nature of freedom of choice and rationality—is addressed by Professor Flew with somewhat more caution and subtlety than has been displayed by many of his predecessors. Each of the essays of which *A Rational Animal* is composed attacks the views of one or more other thinkers: the targets range from Hume to Lenin, and one cannot help feeling that many of them, like Skinner and Freud, were so philosophically naïve that they do not require the weight of artillery that Anthony Flew brings to bear. Moreover, his method of proceeding has the serious disadvantage that amidst the smoke of battle, it becomes difficult to discern the position occupied by the author himself. One longs for a clear statement of his own views, and if in what follows I have misinterpreted him, he is at least partly to blame.

He bases much of his argument on our knowledge that we are free to choose: whenever we perform a voluntary action, we know that we could always have acted otherwise had we so chosen. He takes this alleged fact to be irreconcilable with Laplacean determinism which proposes that each state of the Universe is necessarily caused by its antecedent state. The fact that we can make voluntary movements implies that "What there certainly cannot be . . . is an unbroken chain of sufficient physical causes stretching back indefinitely. It would therefore seem that the central nervous system must either be or contain an apparatus of which the total input does not contingently necessitate every element of total output." He acknowledges that this suggestion is made reluctantly and elsewhere he seems to write as though there were no need for this drastic solution, which implies that the matter in our brains

obeys different laws to those currently thought to hold in inanimate matter. As has often been noted, the invocation of Heisenberg's uncertainty principle is of no help as choices are not made at random and therefore cannot themselves be determined by random events at the quantum level.

Flew's own arguments do not in fact seem to necessitate his conclusion. It no more follows from the fact that we feel free to choose that we are free to choose (in the sense that our choice is not determined by the state of our brains) than it follows that someone who is convinced he can grow wings can grow wings. Moreover, to believe that someone might have chosen differently is surely not inconsistent with believing that he would only have done so had he been in a different antecedent psychological state. This is not to deny the difference between voluntary and involuntary acts. It could be argued that we feel free to choose because we have no way of knowing with certainty what we will do until we decide to do it: in order to know in advance, we would have to simulate at time A our state at time B, and the possible effects of undertaking this simulation at time A on our state at time B could not be included in the simulation itself.

Flew concludes that human actions can never be explained and predicted by physiology: he is correct, but for the wrong reasons. We can no more explain human activity in terms of goings-on in individual nerve cells than we can explain how a computer program works in terms of transistors.

To understand how any organised system of matter works, we must develop concepts appropriate to that kind of system. We can understand and predict the hunting behaviour of a system incorporating negative feedback only in terms of control theory not in terms of previously existing physical concepts. Similarly, the scientific explanation of behaviour requires the development of concepts appropriate to understanding the organisation of the brain in relation to the external world: such concepts may not be physical or even physiological, but in as far as they are both precise and render human actions intelligible in a rigorous way, they are nonetheless scientific. Nor does the need to evolve such explanatory concepts suggest that behaviour is undetermined any more than the fact that computer programs cannot be understood in terms of transistors suggests that the outcome of running a program is undetermined.

A small beginning has been made towards developing the requisite explanatory conceptual system within the disciplines of cognitive psychology and artificial intelligence, and it is a pity that philosophers who write about rationality and actions do not examine these disciplines rather than castigating psychoanalysts, who are not scientists, and physiologists, who work at the wrong level of abstraction to be in the business of explaining human actions. □

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Commissure-section studies

The Integrated Mind. By M. S. Gazzaniga and J. E. LeDoux. Pp.168. (Plenum: New York and London, 1978.)

THE rarity of certain kinds of neurological condition is well recognised. Any investigator who obtained access to more than one case of visual or tactile object agnosia or more than one case of cortical blindness or achromatopsia in a lifetime would count himself lucky indeed.

Surgical brain bi-section falls into a related category. Those of us who work outside of the USA will never have the opportunity to examine such patients (except by visiting the USA), because only in America have the fibre pathways connecting the cerebral hemispheres been surgically divided for the relief of epilepsy. Three separate major series (and one smaller series) of commissure-sectioned patients exist. The earliest patients were investigated before modern and subtle tests of brain

behaviour relationships had been devised. The next (Californian) series is associated with the names of R. W. Sperry and J. Bogen. These patients were carefully studied after exceptionally radical commissure-section; but, regrettably, only a proportion of the patients have been described in the literature. The most recent (New England) series is smaller in number and the patients underwent less extensive neurosurgery.

Michael Gazzaniga, the senior author of this book, has a unique advantage: personal experience of both the Californian and New England series of patients. In addition, he is the originator of many important experiments on 'split-brain' monkeys, and he described some of the earlier findings in 1970, in his book *The Bisected Brain*. In the present book we are brought up to date with recent findings from the New England series. The intention is to "... reestablish a basic, sober framework for considering studies on cerebral commissurotomy", as a counter to "... a barrage of popular and overdramatized accounts ... largely written by people who had never seen a patient ... " (p6).

Will the reader (if he is not put off by the title of the new book, or by the statement on p1 that "The corpus callosum, the largest fiber tract in the human brain, contains over 200 million neurons ...") find what is promised? Sadly, no. The majority of observations reported in the book have already been published in the journals. There is no systematic attempt to group the

observations by the extent of surgery, by the duration or amount of pre-surgical pathology, or by the length of time since surgery at the time of testing. Indeed, a large part of chapter 3 is based on the performance of a single patient (P. S.). Yet we read (p85): "In contrast to all the other patients examined in both series is case P. S.", in whom "... we observed an incredible range of language skills in both hemispheres ...". Certainly, the observations forming the basis of chapter 3 were non-linguistic, but no reasons are given why they were not confirmed on other more typical patients from either series.

And yet, in another framework, the book can be recommended. It is essentially an account of the authors' wide-ranging views on such topics as the origins of lateral asymmetries, the role of language, the basis of intelligence and consciousness, the relationship between perception, imagery and memory, and many more such issues. Most of the opinions are original and provocative; and as opinions they are well argued. In due course adequate evidence to support these opinions may be forthcoming. Meanwhile, the book will serve as an intellectual stimulus to a wide range of neuroscientists who are accustomed to making only painfully slow progress in their own laboratories or clinics: given an "integrated mind", what limit is there to our theorising?

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Whale primer

Whale Manual '78. Friends of the Earth. Pp. 152. (FOE: 9 Poland Street, London, 1978.) £2.

WHALES are by now a well established cause célèbre of the conservation movement, popular on television, and almost universally admired for their benign dispositions and mournful singing. So it comes as something of a jolt to digest the idea that whales could produce a sustainable 2 million tonnes of protein annually (some say), worth perhaps some \$690 million with present prices and utilisation patterns. This little book may help to provide the information to help develop a balanced view on whales and their management, although it remains an enigmatic subject in a manner in which the management of herrings or anchoveta is never likely to be.

In many ways this book is quite excellent. It is ideal as a brief, fact-filled primer on whales and the man-

agement thereof, as long as one accepts that the authors have a (tenable) point of view which significantly colours the text. Not that the contents exclude other people's views, but they are dealt with somewhat erratically. For example, there is some lack of understanding of the reality of economic life, allied to a consistent undertone that, really and truly, whales ought not to be considered as a resource at all but as some sort of monument to man's humanity to mammals. This lack of appreciation of economic factors is illustrated in chapter 6 where the justification for discussing the economics of the whaling industry solely on the basis of the Japanese industry and not that of the USSR is partly the fault of a deficiency of data but mainly results from the fact that the same economic arguments apply to both nations; and in any case slaughtered whales are not interested in "the nicety of distinction between state capital and private capital" (p46). Although the whales may not be bothered in the short run, to dismiss this vital distinction in the exploitation

of what is, as FOE indeed rightly stress, an international common property resource, is not only unjustified but is dangerous. Not only do Soviet fleets operate under significantly different economic parameters, but, as again the FOE stress elsewhere, the USSR has much to gain from any curtailment of solely private capitalistic exploitation.

Overall, the book is fairly good value as it is full of the sort of data and arguments that anyone interested in whales would want to see. It suffers from being an update of a 1975 version, which was an update of the 1972 original. By now it has had a total of 17 authors, and accordingly the deliberately simple style is now patchy, frequently staccato and occasionally irritating. Presentation of the data is generally satisfactory, although some still refers only to 1974-75 which is perhaps disappointing. The few illustrations are rudimentary, but the bibliography is appropriate for the context. The stated objective of the book is to spread information to a wider public, and in this it is likely to succeed. Those in a position to make influential decisions, however, will want to study elsewhere too.

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obituary

D. R. Chick

PROFESSOR D. R. CHICK, F.I.E.E., F.Inst.P., F.I.E.R.E., Head of the Department of Electronic and Electrical Engineering at the University of Surrey since 1965, died suddenly at his home in Surrey on 11 June 1978 aged 61.

He was one of the many young scientists in the 1930s who began their working life on the applications of radar, which were so vital to the winning of the war. His experiences in the field in wartime conditions, where it was so essential to impress the young local commanders that the civil scientists were not wasting everybody's time, surely helped him form some of his strongest characteristics: a quick and accurate assessment of people, straight, often blunt speaking, a sound scientific and economic judgment, and not least a strong personal loyalty to his own staff and colleagues. One such experience where the accommodation offered to him and his co-workers fell far below that expected considering their equivalent army rank led to a walk-out, much to the distress of the commanding officer, who to save his own reputation swallowed his pride and begged their return.

Immediately after the war, Chick turned to industrial research, joining the A.E.I. company at the new research laboratory of Aldermaston Court. For 17 years, he was in charge of various nuclear physics activities of the company and made large contributions in several different ways. His group developed and constructed electrostatic generators and a helical particle accelerator for basic physics research, and among much fundamental work he and colleagues measured excited levels in arsenic 75 produced by radiative capture of protons by germanium 74. This led to a patent application in 1957 relating to the production of p-n junctions in germanium or silicon by nuclear transmutation following irradiation with protons. It is significant that today transmutation doping of silicon by neutrons is used for special devices.

Also, with Sir George Thomson, a large section was devoted to the thermonuclear research programme, so important in future fusion reactors, using a torus named Sceptre. In 1957, this group, working closely with a Harwell team possessing a larger torus

named Zeta, announced the production of neutrons from the discharge and subsequently studied some of the instabilities which develop in toroidal gas discharges carrying large currents.

His third major achievement in this period was the installation of a 5 MW pool type research reactor designed and developed by his group in conjunction with A.E.I. John Thompson Ltd. This reactor was the first publicly owned one and received reactor licence no. 1 in the U.K.; it was officially opened by H.R.H. the Duke of Edinburgh in 1959. It was used for research and contract work and two similar reactors were sold by A.E.I. John Thompson, one in Germany and one to the Atomic Energy Authority at Aldermaston.

In the mid-60s, it was appropriate that this wealth of experience in both the civil service and industry should be passed to one of the new universities which resulted from the publication of the Robbins report. When Professor Chick joined the then Battersea College of Advanced Technology as Head of the Department of Electrical and Control Engineering, he had a decided policy that his department would become known for both its research and industrial liaison as well as for its academic achievements.

He grouped his research effort into a few main projects, and his forward thinking is demonstrated by the fact that in 1966 he formed a team to work on ion implantation, then a speculative research area, now a major part of microelectronics technology, and he saw this group grow and become recognised as one of the major ones in the U.K. with a good reputation abroad.

He also formed an industrial electronics group which made the expertise of his academic staff available to industry on a contract research basis, and already the turnover from this venture has exceeded the £¼ million mark. These successes, together with the establishment of a compulsory industrial year for undergraduates, M.Sc. projects sponsored by many firms, several collaborative Ph.D. programmes and industrially supported research fellowships, are a continuing demonstration of his concept of what makes a good academic engineering department in present day conditions—that is, one where its staff are not only scholars but also professional engineers aware of the needs of the industrial world and

of where real wealth is created.

Professor Chick will be sadly missed by his colleagues and friends for his considerable intellectual qualities, his dry humour and his loyalty. Only days before his death, he visited a sick colleague in hospital carrying ice and a radio. The radio was returned as not working, and when told that the reason was that the batteries were in the wrong way round, he beamed and laughingly said, 'Failed the first term's examination!'

K. G. Stephens
T. E. Allibone

Moses Kunitz

THE death of Moses Kunitz on 24 April 1978 at the age of 90 brought to a close the story-book career of a young emigré from the Russian revolution who was to become a leading scientist in the field of protein research.

Kunitz arrived in New York early in the second decade of this century and found employment in the laboratory of Jaques Loeb, the famous experimental cell physiologist at the then Rockefeller Institute. Dr Loeb recognized the value of Kunitz's quick but careful work and encouraged him to pursue his education in the evenings, first at Cooper Union and then at Columbia University where he received his Ph.D. degree in 1923.

When John H. Northrop was appointed to the place left vacant by Dr Loeb's death, he invited Kunitz to remain and continue his study of proteins. This happy, fruitful union lasted until Dr Northrop left for California in 1951.

Kunitz was an acute observer and experimentalist. He learned to know the properties of proteins so well that he could estimate with some accuracy the likelihood of a protein crystallizing just by watching a flocculation during salt or acid precipitation. One example of this ability stands out in my mind. After attempts by other workers to crystallize the toxic protein ricin during World War II had failed, Kunitz was asked to have a try at it. I brought the package of partially purified ricin to him late one afternoon and remained to watch him weigh a quantity of it, dissolve it in dilute buffer, and distribute aliquots into a number of tubes. He then added increasing numbers of tiny drops of acid to the various tubes. As a cloudiness developed

in a few tubes prior to flocculation, he examined them carefully against indirect light, murmuring to himself 'it isn't so bad', and put the tubes in the refrigerator. The next morning crystals of the ricin protein were clearly visible in two of the tubes.

Kunitz's isolation of some 18 enzymes and precursors in crystalline form from animal and plant tissues and yeast, each with evidence of its purity went far to confirm Northrop's thesis that enzymes are proteins.

Although his name is associated primarily with protein crystallization, Kunitz's contributions and capacity were much broader. In one of his least known and yet more important papers, 'The Kinetics and Thermodynamics of Reversible Denaturation of Crystalline Soybean Trypsin Inhibitor' (*J. gen. Physiol.* 1948, 32, 241), Kunitz demonstrated by enzymatic and physico-chemical methods that renaturation of the inhibitor yielded a product indistinguishable from the original protein. Using a kinetic approach to the equilibrium reaction from both sides at different temperatures, he obtained clean thermodynamic values for each process. This study laid to rest the prevailing notion that so complicated a process as protein denaturation could not be completely reversible.

On the occasion of Kunitz's being awarded the Carl Neuberg Medal in 1957 by the American Society of European Chemists and Pharmacists, Dr Northrop wrote the following: 'Dr Kunitz possesses to a rare degree the abilities of a research worker of the first rank in his chosen field—imagination, ingenuity, and persistence, great technical skill, mathematical facility and thorough theoretical knowledge. It is not surprising, therefore, that he has been able to solve almost every problem he has attempted. Some of them are of great importance. The isolation and crystallization of ribonuclease, hexokinase, and desoxyribonuclease placed the protein nature of enzymes in general on firm experimental foundation. In addition, the nucleases have been invaluable tools in the elucidation of the chemistry of the nucleic acids, those remarkable substances that appear to be the very "stuff of life".'

Kunitz was the first to demonstrate that certain enzymes, especially the proteases of mammals, existed in glandular tissues in an inactive form. This formation explained why the tissue proteins were not digested. He went on to study the autocatalytic or catalytic conversion of these precursors to active enzymes following secretion into the digestive fluids.

To present-day biomedical investigators Kunitz may be remembered for having purified the pancreatic nucleases, ribonuclease and deoxyribonuclease which, as Dr Northrop has noted, have become and will continue to be very important in identifying the functions of the various nucleic acids.

During his lifetime, Dr Kunitz received a number of honours including the Carl Neuberg Medal and membership in the National Academy of Sciences. He was made a full member of the Rockefeller Institute in 1950 and became a Professor when the Institute became a University.

Moses Kunitz is survived by a son, Jaques, and a daughter, Rosaline Albert.

Roger M. Herriott

R. K. Murton

RON MURTON was one of the few really professional scientific ornithologists, who combined careful and patient field observation of birds with rigorous scientific investigation on their anatomy and physiology. His death from a heart attack on 12 June 1978, at the early age of 46, came as a great shock to his colleagues and friends. Although he had a previous attack some weeks earlier, and had been seriously ill, he had returned to part-time work with his enthusiasm undiminished, and he appeared to be making a good recovery. Up till this spring he had always had such energy and drive, and the ability to work at high pressure sparing neither himself nor his colleagues, that we had never imagined that he would ever suffer from ill health.

Ronald Keir Murton was born in 1932. The first sixteen years of his working life, after graduating in Zoology at University College London, were with the Infestation Control Division of the Ministry of Agriculture, Fisheries and Food. He became immediately involved in the study of birds which are agricultural pests, and with the wood pigeon in particular. The damage done by wood pigeons to brussels sprouts and other brassicae, amounting to many millions of pounds every year, was well known, and the main attempt at control was by supplying cheap cartridges to enable farmers and others to shoot the birds. This gave much pleasure to these sportsmen, but, as Murton soon showed, it had little significant effect on pigeon numbers and it did not prevent the birds from doing economic damage. As a result of his findings the cheap cartridge scheme was stopped, saving the taxpayer a considerable sum of money; farmers whose sport became more expensive were less enthusiastic.

Murton's work on pigeons had a very practical aim, and it was successful in identifying the problems even if it was not possible to find any easy solution. Although the study was what would today be called 'customer orientated', it was also (as such studies may be) work of real scientific quality. His observations on pigeon behaviour

and reproductive physiology have seldom been equalled. The results are recorded in some 30 scientific papers, in a 'New Naturalist' monograph volume, and they formed the subject of the Barnes Memorial Lecture, which he was invited to give to the Association of Applied Biologists at the unusually early age of 32.

Although Dr Murton's career was almost entirely in research, he was closely associated with the academic world. In 1968–69 he enjoyed a year's sabbatical leave as a visiting lecturer at the University of Hong Kong. In 1974 he was appointed an Honorary Reader in the department of zoology at Hull University, and recently he was promoted to Honorary Professor. He was in demand to lecture to university students and others.

After leaving Hong Kong he returned briefly to the Ministry of Agriculture in 1969, and then transferred to Monks Wood Experimental Station, at that time under the Nature Conservancy. When the research branch of the Conservancy was reorganised as the Institute of Terrestrial Ecology he remained at Monks Wood, and was soon appointed as Head of the Sub-Division of Animal Function.

During the years when he was working on pigeons, which included important observations on feral pigeons in cities, Murton realised the importance of studies of the physiological changes in these birds as a basis for understanding their behaviour and their whole ecology. He became much involved in such topics as the photo-periodic control of the breeding cycle, and other, mainly laboratory, studies. He considered that these were the logical development of his earlier work. Many colleagues regretted his virtual abandonment of those field studies at which he had been so adept, and thought him ill advised to attach so much importance to his newer investigations. Not all his recent work has had the general approval given to his outstanding pigeon studies. Only time would have shown whether his switch in interest was, as he insisted, wise.

As well as observing birds in the field, Murton was one of the most outstanding bird photographers. As long ago as 1963 he was elected to Association of the Royal Photographic Society. During his comparatively short period, just twelve months, in Hong Kong, he made a surprisingly large collection of slides of the nesting habits of many of the rarer and more interesting species, several of which had not been previously photographed. He used his own photographs to illustrate his books and his lectures.

Kenneth Mellanby

announcements

Awards

The Fields Medal has been awarded to **Dr Daniel G. Quillen**, (Massachusetts Institute of Technology) for his work on algebraic K-theory.

The first recipient of the C. D. Nelson Award of the Canadian Society of Plant Physiologists, is **Dr J. Derek Bewley** (University of Calgary) for his research contributions to plant physiology.

The winners of the Filtration Society's 1978 Travel and Study awards are: **G. H. Read** (Imperial College, London) and **Maureen Farrell** (Georgia). **Professor Frank Tiller** (University of Texas) has won the Society's gold medal for his work on delayed cake filtration.

Henry F. Schaeffer III (University of California, Berkeley) is the 1979 recipient of the ACS Award in Pure Chemistry.

Professor G. Vassart (University of Brussels) has been awarded the bi-annual 'Sir Charles Harrington Prize' by the European Thyroid Association for his work on the thyroglobulin messenger RNA.

The RIC have awarded the 1977 Meldola Medal and Prize to **Professor Eric Oldfield** (University of Illinois) for his work on the NMR spectroscopy of biochemical systems.

The Marlow Medal is restricted to Fellows of the Faraday Division of the Chemical Society of not less than 3yr standing on the closing date for application (1 January 1979). Applicants should be under 33 at this date. The award is based on publications on any subject normally published in JCS Faraday Transactions I and II. Applications to: Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK).

Appointments

Professor Barbara E. Clayton to the Chair of Chemical Pathology and and Human Metabolism at the University of Southampton.

Professor James R. Gray to Dean of the Faculty of Science at Heriot-Watt University.

Dr Asa G. H. Blakeley to the Chair of applied physiology at the University of Leicester.

Professor Peter G. Sammes to the Chair of Organic Chemistry at Leeds University.

Charles E. M. Yates to be Professor of Mathematical Logic at The University of Manchester.

Geoffrey Edwards to be chairman of the Thames Water Authority.

Dr Michael I. Gurr to head of the NIRD's Nutrition Department.

Meetings

25-30 November, **4th World Ozone Congress**, Houston (Dr A. Netzer, Environmental Sciences, University of Texas at Dallas, Richardson Texas 75080).

27 November-1 December, **Radiation Protection in Nuclear Power Plants and the Fuel Cycle**, Bristol (BNES, 1-7 Great George St, Westminster, London SW1, UK).

27-30 November, **Forage Conservation in the '80's**, Brighton (The Secretary, The British Grassland Society, Grassland Research Institute, Hurley, Maidenhead, Berks, UK).

28-30 November, **International Conference Antennas and Propagation**, London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

30 November-1 December, **Cell Surfaces and Neoplasia**, London (Dr M. Moore, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Manchester, UK).

30 November-1 December, **Symposium on Nutrition and Gastroenterology**, New York (Institute of Human Nutrition, Columbia University, College of Physicians and Surgeons, 701 West 168th St, New York, New York 10032).

1-2 December, **Current Concepts of Pain and its Treatment**, New York (Dr R. Duvoisin, ARNMD, Annesberg Building, Suite 14-02, 100th St at 5th Ave, New York, New York 10029).

2-3 December, **Science and Art in the 14th Century**, New York (Ann Collins,

The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

4-6 December, **3rd Annual Toxic Substances Control Conference**, Washington (Government Institutes Inc., 4733 Bethesda Ave, Washington, D.C. 20014).

6-7 December, **Engineering and the Environment: Harmony or Conflict?**, London (The Secretary, The Institution of Water Engineers and Scientists, 6-8 Sackville St, Piccadilly, London W1, UK).

7 December, **Structure Analysis for Surfaces**, York (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

11-14 December, **Design of Hydrologic Data Networks**, Tuscon (Meetings AGU, 1909 K St, Washington, D.C. 20006).

13 December, **Microstructural Instability: its influence on long service life**, Birmingham (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

14-19 December, **9th Texas Symposium on Relativistic Astrophysics**, Munich (Prof. J. Ehlers, MPI, Foehringer Ring 6, D 8000 München 40, FRG).

18-19 December, **Pulsed NMR in Solids**, London (Prof. J. A. S. Smith, Chemistry Dept, Queen Elizabeth College, Campden Hill Rd, London W8, UK).

19-21 December, **Enzyme Active Sites**, London (Dr H. C. Watson, Dept of Biochemistry, University of Bristol Medical School, Bristol, UK).

3-5 January 1979, **16th Annual Solid State Physics Conference**, Warwick (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

4-5 January, **Laser Plasma Interactions**, Bangor (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

8-12 January, **11th Miami Winter Symposium**, Miami (UMED-PO Box 520875, Miami, Florida 33152).

14–16 January, **5th Annual Meeting of the International Embryo Transfer Society**, Denver (Mrs S. Seidel, IETS, 3101 Arrowhead Road, LaPorte, Colorado 80535).

15–18 January, **2nd International Colloquium on Mars**, Pasadena (Dr C. W. Snyder, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, California 91103).

22–23 January, **Medical and Industrial Applications of DNA**, Chicago (Robert S. First Inc., 405 Lexington Ave, New York, New York 10017).

5–9 February, **International Conference on Neurotoxins**, South Australia (Dr I. W. Chubb, Dept of Human Physiology, Flinders Medical Centre, Bedford Park, South Australia 5042, Australia).

12–13 February, **Medical and Industrial Application of DNA**, London (Robert S. First Inc., 405 Lexington Ave, New York, New York 10017).

12–24 February, **World Climate Conference**, Geneva (WMO, Ave Giuseppe Motta 41, Geneva, Switzerland).

13–15 February, **Use of the Microprocessor as a Component in Measurement and Control Equipment**, Stevenage (Carole Meads, Sira Institute, South Hill, Chislehurst, Kent, UK).

21–24 February, **Hypoxia**, Banff (Arctic Institute of North America, University Library Tower, 2920–24th Ave N.W. Calgary, Alberta, Canada T2N1N4).

5–9 March, **30th Annual Conference on Analytical Chemistry and Applied Spectroscopy**, Cleveland (Mrs L. Briggs, 437 Donald Rd, Pittsburgh, Pasadena 15235).

12–14 March, **Controlled Environments Working Conference**, Madison (T. W. Tibbitts, Dept of Agriculture, University of Wisconsin-Madison, 1575 Linden Drive, Madison, Wisconsin 53706).

12–15 March, **International Cashew Symposium**, Cochin (Central Plantation Crops Research Institute, Kasaragod 670 124, Cannoanore District, Kerala, India).

12–16 March, **Tunnelling '79**, London (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

14–16 March, **Thermal Characteristics of Tumors: Applications in Detection and Treatment**, New York (Conference Dept, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

14–23 March, **Einstein Centenary in Jerusalem** (Israel Academy of Sciences and Humanities, PO Box 4040, Jerusalem, Israel).

26–29 March, **2nd European Conference on Surface Science**, Cambridge (Dr P. M. Williams, c/o VG Scientific Ltd, The Birches Industrial Estate, East Grinstead, Sussex, UK).

27 March–6 April, **7th Edmond de Rothschild School in Molecular Biophysics on the Structure of Chromatin**, Rehovot (Dr H. Eisenberg, Weizmann Institute of Science, Rehovot, Israel).

29–30 March, **International Conferences on Spectroscopy**, Miami (Vijay Mohan Bhatnagar, Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

1–8 April, **International Ophiolite Symposium**, Nicosia (Chairman, Geological Survey Department, Nicosia, Cyprus).

2–4 April, **Vacuum Processes in the Electronics Industry**, Brighton (Carole Meads, Sira Institute, South Hill, Chislehurst, Kent, UK).

4–6 April, **International Conferences on the History of Museums and Collections in Natural History**, London (Mrs J. A. Diment, Palaeontology Library, British Museum (Natural History), Cromwell Road, London SW7, UK).

3–5 April, **Molecular Dynamics of Polymeric Systems**, Cambridge (Dr C. W. Smith, Secretary Dielectrics Society, Dept of Electrical Engineering University of Salford, Salford, UK).

9–11 April, **Kinetics of State, Selected Species**, Birmingham (Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK).

9–12 April, **11th National Atomic and Molecular Physics Conference**, Liverpool (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

17–20 April, **Electrostatics 1979**, Oxford (Dr J. F. Hughes, Dept of Electrical Engineering, University of Southampton, Southampton, UK).

24–25 April, **Advances in Optical Production Technology**, Teddington (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

29 April–2 May, **Protein-nucleic acid interactions**, Sitges (Prof. J. A. Subirana, Departamento de Quimica Macromolecular del C.S.I.C. Escuela T.S. de Ingenieros Industriales, Barcelona (28), Spain).

30 April–2 May, **Learning to use our Environment**, Seattle (Dr A. Roffman, Energy Impact Associates Inc., PO Box 1899, Pittsburgh, Pasadena 15230).

30 April–3 May, **International Conference on Malaria and Babesiosis**, Mexico (Dr J. P. Kreier, College of Biological Science, The Ohio State University, Columbus, Ohio 43210).

2–3 May, **Monitoring Toxic and Flammable Cases and Vapours**, London (Mrs R. Keiler, Sira Institute, South Hill, Chislehurst, Kent, UK).

19–20 April, **2nd Canadian Chromatography Conference**, Toronto (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

3–4 May, **6th International Symposium on Flammability and Fire Retardants**, Nashville (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

7–11 May, **1st International Colloquium on Receptors, Neurotransmitters and Peptide Hormones**, Capri (F. Fraioli, Patologia Medica II, Policlinico Umberto I, Università, 00161 Rome, Italy).

15–18 May, **The Agricultural Industry and its effects on Water Quality**, Hamilton (Dr C. J. Schouten, Water and Soil Division, Ministry of Works and Development, Private Bag, Hamilton, New Zealand).

21–23 May, **Television Measurement**, London (Conference Secretariat, IERE, 99 Gower St, London WC1, UK).

21–23 May, **10th International Symposium on Chromatography and Electrophoresis**, Rimini (Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche, Via Eritrea, 62-20157 Milan, Italy).

21–25 May, **Colloids and Surfaces**, Pittsburgh (Mrs G. Cohen, Carnegie Institute of Technology, Carnegie-Mellon University, Schenley Park, Pittsburgh, Pennsylvania 15213).

27 May–1 June, **14th World Gas Conference**, Toronto (Public Affairs Committee, 111 St Clair Ave West, Toronto, Ontario, Canada).